SUGAR CHEMISTRY

SUGAR CHEMISTRY

other AVI books

Food Chemistry

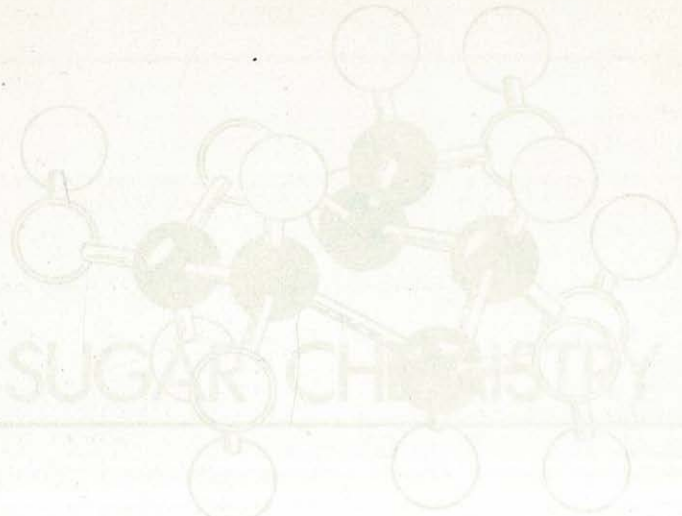
FISH OILS *Stansby*
FOOD ANALYSIS LABORATORY EXPERIMENTS *Meloan and Pomeranz*
FOOD ANALYSIS: THEORY AND PRACTICE *Pomeranz and Meloan*
FOOD CHEMISTRY *Aurand and Woods*
FOOD OILS AND THEIR USES *Weiss*
FUNDAMENTALS OF DAIRY CHEMISTRY, 2ND EDITION *Webb, Johnson, and Alford*
PHOSPHATES IN FOOD PROCESSING *DeMan and Melnychyn*
SYMPOSIUM: SWEETENERS *Inglett*

Food Science and Technology

ECONOMICS OF FOOD PROCESSING *Greig*
ECONOMICS OF NEW FOOD PRODUCT DEVELOPMENT *Desrosier and Desrosier*
EGG SCIENCE AND TECHNOLOGY *Stadelman and Cotterill*
FOOD AND THE CONSUMER *Kramer*
FOOD DEHYDRATION, 2ND EDITION, VOL. 1 AND 2 *Van Arsdel, Copely, and Morgan*
FOOD FOR THOUGHT *Labuza*
FOOD SCIENCE, 2ND EDITION *Potter*
FREEZING PRESERVATION OF FOODS, 4TH EDITION, VOL. 1, 2, 3, AND 4 *Tressler, Van Arsdel, and Copley*
HANDBOOK OF SUGARS *Junk and Pancoast*
LABORATORY MANUAL FOR FOOD CANNERS AND PROCESSORS, 3RD EDITION, VOL. 1 AND 2 *National Canners Association*
MICROBIOLOGY OF FOOD FERMENTATIONS *Pederson*
PRACTICAL FOOD MICROBIOLOGY, 2ND EDITION *Weiser, Mountney, and Gould*
PRINCIPLES OF PACKAGE DEVELOPMENT *Griffin and Sacharow*
PROCESSED MEATS *Kramlich, Pearson, and Tauber*
PROGRESS IN REFRIGERATION SCIENCE AND TECHNOLOGY, VOL. 1, 2, 3, AND 4 *Pentzer*
QUALITY CONTROL FOR THE FOOD INDUSTRY, 3RD EDITION, VOL. 1 AND 2 *Kramer and Twigg*
SEED PROTEINS: A SYMPOSIUM *Inglett*
STORY OF FOOD *Garard*
TECHNOLOGY OF FOOD PRESERVATION, 3RD EDITION *Desrosier*
YEAST TECHNOLOGY *Reed and Peppler*

Nutrition and Biochemistry

CARBOHYDRATES AND THEIR ROLES *Schultz, Cain, and Wrolstad*
CHEMISTRY AND PHYSIOLOGY OF FLAVORS *Schultz, Day, and Libbey*
FOOD ENZYMES *Schultz*
LIPIDS AND THEIR OXIDATION *Schultz, Day, and Sinnhuber*
NUTRITIONAL EVALUATION OF FOOD PROCESSING *Harris and Von Loesecke*
PROGRESS IN HUMAN NUTRITION, VOL. 1 *Margen*
PROTEINS AND THEIR REACTIONS *Schultz and Anglemier*
PROTEINS AS HUMAN FOOD *Lawrie*
SELENIUM IN BIOMEDICINE *Muth, Oldfield and Weswig*
SULFUR IN NUTRITION *Muth and Oldfield*

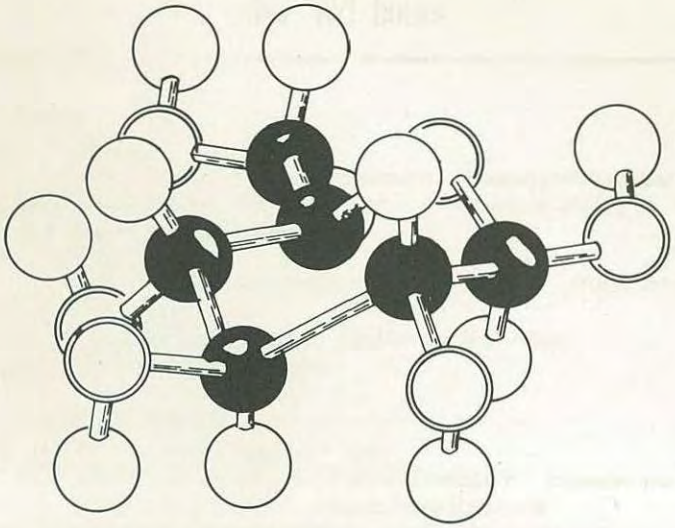


Can ye make a model of it? For if ye can, ye understand it, and if ye canna, ye dinna!

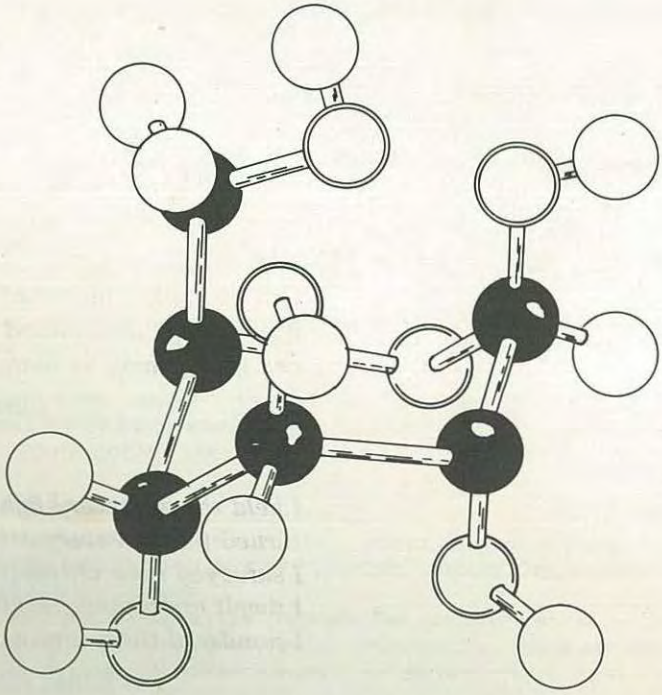
Lord Kelvin

I held them in every light. I turned them in every attitude. I surveyed their characteristics. I dwelt upon their peculiarities. I pondered their conformation.

Edgar Allen Poe



THE FAVORED CI CONFORMATION OF β-D-GLUCOSE



THE IC CONFORMATION OF β-D-GLUCOSE

SUGAR CHEMISTRY

by R. S. SHALLENBERGER
Professor of Biochemistry,
New York State Agricultural
Experiment Station,
Cornell University,
Geneva, New York,
United States of America

and G. G. BIRCH
Lecturer, National College
of Food Technology,
Reading University, Weybridge,
Surrey, United Kingdom

WESTPORT, CONNECTICUT
THE AVI PUBLISHING COMPANY, INC.
1975

© *Copyright 1975 by*
THE AVI PUBLISHING COMPANY, INC.
Westport, Connecticut

All rights reserved. No part of this work covered by the copyright hereon may be reproduced or used in any form or by any means—graphic, electronic, or mechanical, including photocopying, recording, taping, or information storage and retrieval systems—without written permission of the publisher.

Library of Congress Catalog Card Number: 74-84467
ISBN-0-87055-166-3

Printed in the United States of America

QD
321
.S43

To
Carol Naumann Shallenberger
and
Kathleen Martin Birch

248774

QP
351
.S43

248774

Foreword

The continued active interest in the chemistry of the carbohydrates has provided a wealth of knowledge concerning their detailed structures, their derivatives and their modes of reaction. One of the most significant features of this work has been a realization of the importance of the geometrical conformation of a sugar molecule as a factor in determining the type of chemical reaction it will undergo. These considerations are now known to be of particular importance in biochemical aspects of carbohydrate chemistry as well as in many industrial applications of sugar chemistry, including food technology. In this volume, the authors have provided a succinct account of the fundamental chemistry of the sugars, with special attention to those properties, an understanding of which is necessary in dealing with applications of sugars in fields such as agriculture, botany, medicine, food science and technology. Emphasis has rightly been placed on conformational aspects, and the basic organic chemistry of the sugars has been dealt with in terms of conformational theory. The way is then open for concise, but authoritative chapters dealing with various applications of carbohydrates in biochemistry, medicine and technology. These include accounts of the metabolism of sugars and the part they play in nutrition. Special attention is devoted to problems of taste as exhibited by sugars and their derivatives. In view of their importance in the food industry, browning reactions are discussed at length, and brief accounts are given of fermentation phenomena and of acidic and enzymic hydrolysis of starches in the preparation of glucose syrups. Much of this information is not readily available to the general reader, who will find in the clearly written text, the numerous diagrams and tables and the wealth of references a most useful guide to these important aspects of carbohydrate chemistry.

E. L. HIRST

May 1974
Edinburgh

Preface

This collaborative effort is written primarily for those persons interested in the chemistry of the sugars with direction toward their use in foods. The subject matter emphasizes the *intrinsic* chemistry of the sugars from the viewpoint of the chemical and physical properties exhibited by the sugars themselves. In this sense we feel that the subject matter has much more extensive and general application.

In the introductory and second chapters, the structures of the sugars are presented in the various forms known today. By applying conformational principles we have attempted to present these structures in such a manner as to illustrate the way structure predetermines and directs chemical and physical properties. It seems to us that this is one of the fascinating areas of carbohydrate chemistry, and that such studies will lead to an ultimate understanding of the intriguing chemical and physical properties of the sugars.

In addition to their great biological importance, the sugars serve as suitable models for the study of stereochemistry. Professor S. J. Angyal at the University of New South Wales has again pointed out that proof of the soundness of the tetrahedral theory of the nature of the carbon atom was presented by Emil Fischer by establishing the configuration of the monosaccharides. In recognition of the variety of shapes possible for the ring forms of the sugars, Sir Norman Haworth first introduced the stereochemical term *conformation* to organic chemistry. Van't Hoff's discovery of the relation between the osmotic properties of the sugars and the gas laws contributed greatly to the formalization of the subject of physical chemistry.

Unfortunately the need to present the structures of the sugars in the many ways in which they can exist in order to explain an organic chemical, biochemical, or stereochemical phenomenon seems to cause some prejudice against the subject, and may indeed cause it to appear intractable. The ability of the sugars to assume many forms is, however, intrinsic, and we have attempted to present the various structures of the sugars in Chapters 1 and 2 in such a way as to generate the opposite effect.

In Chapter 3 the general distribution of sugars in nature is presented, together with some important fundamental chemical and physical properties. Not only are these properties essential to an understanding of the structures of the sugars, but they also lead to

that knowledge of their intrinsic chemical behavior that is of special importance to food chemistry.

Chapter 4 could also be entitled "The Chemistry of Sugars in Aqueous Solution." Emphasis is placed upon the mutarotation, enolization and isomerization reactions, and these reactions, as indicated by the studies of H. S. Isbell and W. W. Pigman, are the key to the discovery of further knowledge of the unique properties of sugars.

Some nutritional aspects of the sugars are discussed in Chapter 5. It will be obvious to the reader that we consider the sweetness of the sugars to be a very basic nutritional attribute, and champion the thesis that sugar sweetness can be related to the stereostructure and the intrinsic chemical properties of the sugars, as is also true of all other biological properties. Additional information on the role of sugars in food and medicine is presented in Chapter 6.

Chapter 7 is devoted to the so-called nonenzymic browning reactions of the sugars because of their importance to the color, flavor, texture and nutritive value of foods; further progress in the chemistry of this "nebulous" group of reactions depends on the understanding and progress in the areas of sugar chemistry presented in earlier chapters. The final chapter discusses the hydrolysis of oligosaccharides and the fermentation of sugars to produce food products.

Many of our colleagues have had great influence on our understanding of the structure and reactions of the sugars. Although they cannot be named without omission, we express our gratitude to Sir Edmund Hirst, Horace Isbell, Melville Wolfrom, J. K. N. Jones, R. U. Lemieux, Nelson K. Richtmyer, John Hodge, Derek Horton, and R. Stuart Tipson.

Some of the data presented are heretofore unpublished results obtained in our laboratories and are now introduced to "round out" a discussion. One example is the data obtained by C. Y. Lee and T. E. Acree on fructose mutarotation.

Many of the drawings, graphs and cuts were prepared by Monica Juffs and Cynthia Searles. We are grateful to them.

R. S. SHALLENBERGER,
Geneva, New York

G. G. BIRCH
Weybridge-Surrey

January 1974

Contents

CHAPTER		PAGE
1.	INTRODUCTION TO THE INTRINSIC CHEMISTRY OF SUGARS	1
2.	STRUCTURE OF MONOSACCHARIDES	17
3.	OCCURRENCE AND PROPERTIES OF SUGARS	46
4.	INTRINSIC CHEMICAL REACTIONS OF SUGARS	89
5.	NUTRITIONAL AND METABOLIC ASPECTS OF SUGARS	113
6.	ROLE OF SUGARS IN FOOD AND MEDICINE	147
7.	NONENZYMIC BROWNING REACTIONS	169
8.	ENZYMIC HYDROLYSIS OF OLIGOSACCHARIDES, AND FERMENTATION	194
	INDEX	207

Introduction to the Intrinsic Chemistry of Sugars

THE BIOGENESIS OF SUGAR

The process by which the energy of the sun is captured for subsequent utilization for the growth and maintenance of life is called photosynthesis. In the process, carbon dioxide and water are taken up by the green plant and converted, in the presence of light, to carbohydrate (carbon plus water, or "carbon hydrates"). The classic equation for the process is:

$$CO_2 + H_2O^* \xrightarrow[\text{light}]{} CH_2O + O_2^*$$

and photosynthesis may be defined as "the reduction of carbon dioxide to carbohydrate" since the hydrogen component of water is used to reduce the CO_2 and the oxygen component is evolved as a gas.

The major product of photosynthesis is the 6-carbon sugar D-glucose, or polymers of D-glucose. Plants can synthesize their other compounds from sugar and minerals. Thus, the empirical equation for photosynthesis can be written as a molecular equation:

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

and the energy stored in one mole of a hexose sugar is about 675 Calories.

The molecular photosynthetic equation was elegantly confirmed by Smith (1949). As shown in Fig. 1.1, the ratio of carbon dioxide absorbed to carbon stored as carbohydrate (in excised leaves) is nearly unity; the general molecular photosynthetic equation is not strictly true, however, particularly when plants are growing rapidly. Carbon compounds smaller than hexoses may enter directly into reactions which synthesize other essential compounds such as amino acids, lipids, and hormones. All organic compounds are ultimately interrelatable through metabolic pathways.

The energy contained in the various compounds synthesized can be biochemically released through reverse reactions such as respiration and glycolysis. In any event, the bulk of the energy captured by photosynthesis is accumulated and stored in sugars or sugar polymers.

CONSTITUTION OF GLUCOSE

The keystone carbohydrate is the sugar D-glucose; other names for this compound are *grape sugar*, *starch sugar*, and *dextrose*. The latter

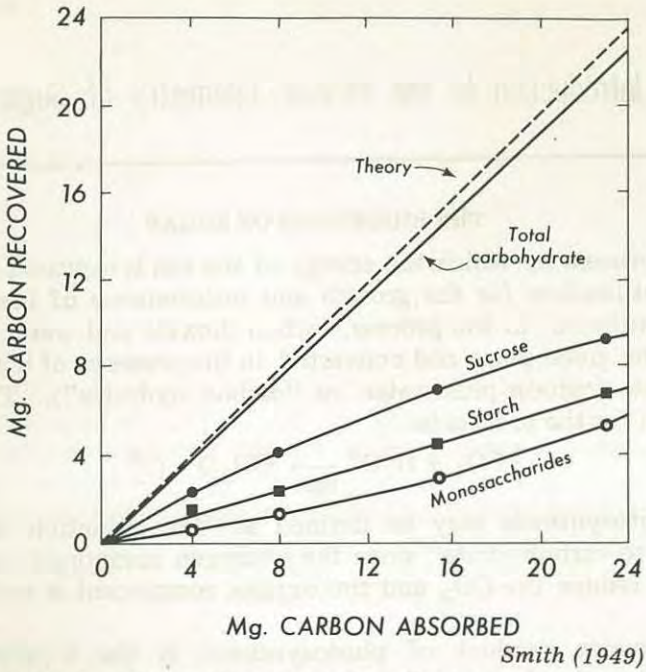


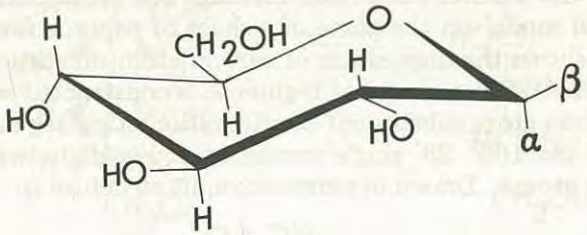
FIG. 1.1. DEPOSITION OF CARBON, FROM CO_2, AS CARBOHYDRATE IN EXCISED LEAVES DURING PHOTO SYNTHESIS

term is used to describe the substance as it is available on a commercial scale, or from chemical supply houses. D-Glucose is a unique compound. It can exist in a variety of structures, which is one of the reasons why it is shown structurally in several different ways. Usually the method chosen to depict the structure is selected to emphasize a special property or reaction. This versatile compound can participate in many chemical reactions, and in different environments can proceed, through different chemical mechanisms and pathways, to yield the same end products.

One form of D-glucose, α-D-glucopyranose, is the usual crystalline form, and the repeating unit of the polymer starch and glycogen (animal starch). Another form, β-D-glucopyranose, is the sugar usually found in glycosidic union in plant pigments. It is also the repeating monomeric unit of cellulose, the structural substance of plants; it is the only form of the compound acted upon by the enzyme glucose oxidase. Still another important form of D-glucose has a free aldehyde group, which gives it characteristic reducing properties in solution. The most common forms of D-glucose have a puckered 6-membered heterocyclic ring.

Conformational Formula of D-Glucose

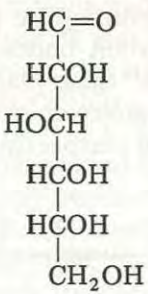
The structural formula for the usual form of D-glucose is:



The formula is drawn in perspective to show the favored shape (conformation) of the 6-membered ring form. Only the thin-line portion of the ring structure is viewed as being drawn on the plane of the paper; the thick front edge of the ring is projected directly toward the viewer to indicate that it is not actually on the plane of the paper. The Greek letter alpha indicates the position of a lone hydroxyl group on the anomeric carbon atom for the sugar α-D-glucopyranose. If this hydroxyl group occupies the other position, the sugar is β-D-glucose. Thus, D-glucose is actually two distinctly different sugars, but they are so closely related, being configurational isomers at an anomeric center, that merely dissolving the crystalline form of either of them in a suitable solvent gives a solution of the two forms in equilibrium with each other. In the structure shown above, the D-glucoses can also be considered to be labile hemiacetals.

Fischer Projection Formula

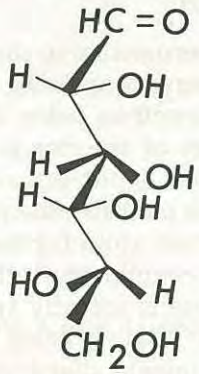
The aldehydo formula for D-glucose is



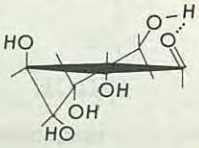
and the method of depicting this is known as the *Fischer projection*. The formula is easily printed on the plane of a sheet of paper, and clearly shows that the sugar is an aldehyde with many of the chemical properties and reactions of an aldehyde. The structure also clearly shows the configuration, now known to be absolute, about each asymmetric carbon atom. The Fischer proof of this structural

form of D-glucose is a classic example of deductive chemical reasoning (Hudson 1941).

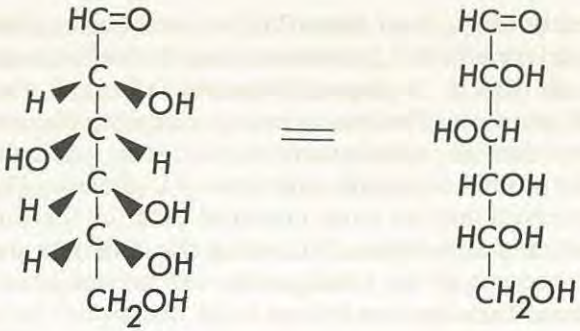
Because the Fischer projection formula is a projection of a three-dimensional model on the plane of a sheet of paper, a few comments on how it shows the disposition of carbon atom substituents in space are in order. When a model of D-glucose is constructed with the preceding carbon atom substituent configuration, a zig-zag model results because of the 109° 28′ single covalent bond angle between contiguous carbon atoms. Drawn in perspective, its structure is:



The heavy darts indicate that the carbon atoms or their substituents are projected outward from the plane of the paper. Thin lines drawn obliquely indicate atoms which project back, through the plane of the paper. This perspective drawing for the normal chain of D-glucose seems to bear no relation to the Fischer projection formula for D-glucose, nor to the projection formula of any other aldohexose, for that matter. By restructuring the model by rotation about the single covalent carbon-to-carbon bonds, a *pseudo* 7-membered ring can be formed, and "closure" takes place between the carbonyl oxygen atom and the hydroxyl group on the terminal carbon atom. The following structure, shown in perspective, then results:



Starting with the carbonyl group and viewing the following carbon atoms by rotating the pseudo ring leads to a linear unfolding of the ring in which all asymmetric carbon atom substituents project toward the reader. As shown next, this is precisely what the Fischer projection formula signifies.



It is often stated that one shortcoming of the projection formula is that it does not show the stereodisposition of carbon-atom substituents in relation to each other; but this can hardly be expected of a formula which serves to emphasize *individual* carbon-atom configuration and the fact that one of the forms is believed to be acyclic. On the other hand, the fact that glucose can be oxidized to give a normal acid and reduced to a normal alcohol is proof that glucose, regardless of favored structure, can yield normal alcohols and acids upon being reduced or oxidized.

Development of the Composition and Formulas for Glucose

The accepted conformational and normal structures of D-glucose are given credence by detailing major features of the key studies that led to the present position. Table 1.1 shows the various levels of attainment reached by investigators in their attempt to unravel the structure and composition of this compound.

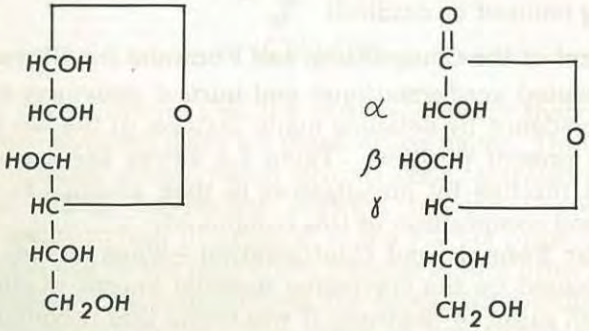
Molecular Formula and Configuration.—When elemental analyses were conducted on the crystalline material known as glucose, grape sugar, starch sugar, or dextrose, it was found that it contained carbon and also hydrogen and oxygen, the latter two elements being in the same proportion as in water (2H/O). As a result, the empirical formula for glucose was described as *hydrate de carbone* in French and *kohlenhydrat* in German. This led to the English term *carbohydrate*.

Tollens (1883) deduced, by application of the findings of Baeyer (1870) and Fittig (1871) to the results he had obtained, that the aldehyde-like sugar, glucose, has the molecular formula $C_6H_{12}O_6$, even though suitable methods for determining the molecular weight of a compound were not then known. Tollens also deduced that the sugar has a cyclic structure. The studies of Baeyer and Fittig were also known to Kiliani (1886), who proved that upon cyanohydrin addition, glucose gave a normal-chain 7-carbon acid. Kiliani's work also confirmed the aldehydic properties of glucose and showed the molecular formula to be $C_6H_{12}O_6$. The molecular formula for glu-

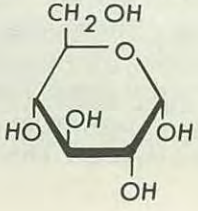
cose was firmly established when Tollens and Mayer (1888) applied Raoult's "gefriermethode" for determining molecular weights.

Through an elegant display of chemical technique, deductive reasoning, and intuition, Fischer, as mentioned earlier, established the configuration for all substituents about each asymmetric carbon atom for the normal aldehydo structure of D-glucose. His configurational assignments derived from chemical data for the three consecutive asymmetric carbon atoms following the aldehyde group, and his intuitive assignment of the configuration about the penultimate (reference) carbon atom are now known to be absolute.

Ring Structures.—When more than one crystalline isomeric form of D-glucose became known (Tanret 1895), it became clear (Armstrong 1903; Hudson 1909) that the postulated ring form for D-glucose must exist, since the additional asymmetric center needed to account for this fact could be brought about only by formation of a cyclic structure. Through analogy with the stable structure of the known γ lactones, it was assumed that the γ-lactol ring was the probable structure for the sugars.



The useful rotation rules of Hudson (1909) and the chemical proof of the configuration about the new asymmetric carbon (Böeseken 1913) were accomplished by using this presumption. Later studies by Hirst and Purves (1923), Drew and Haworth (1926) and Charleton *et al.* (1926) resulted in strong evidence that the aldopentoses and hexoses must usually possess a 6-membered amylene oxide (delta-lactol) ring. These findings led to depicting the structure of the sugars in a perspective pyranoid form (Haworth 1928).



It now seems that exo double bonds, such as those possessed by the lactones, stabilize a 5-membered ring, but they are an element of instability on a 6-membered ring (Brown *et al.*, 1953). Therefore, the sugar lactones with an exo double bond (C=O) have the 5-membered ring as the favored structure, but the 6-membered hemiacetal sugar ring with no exo double bond is the favored structure for the sugars themselves.

In the Haworth three-dimensional formula, the lower edge of the ring is projected toward the reader, while the back edge of the ring is on the plane of the paper. The C—C bond angles of the 6-membered ring are 109° 28′, and the ring cannot be strictly planar as shown, but must be puckered. Haworth (1928) subsequently suggested a number of forms (conformations) for the 6-membered ring. Isbell (1937) later used the idea of pyranoid ring conformations to explain the various rates shown by sugar anomers when subjected to oxidation by bromine.

Ring Conformations.—Hassel and Ottar (1947) and Reeves (1949) laid the foundation for determining the various shapes the sugar molecule may assume, whether at "rest" in a "favored" conformation, or when participating in a chemical reaction. Some of these conformations are shown in Table 1.1. These are also perspective formulas depicting three-dimensional structures of glucose. Detailed discussion of these structures will be given in the following chapter, but some of the means of specifying conformation are given in the following discussion of sugar nomenclature.

NOMENCLATURE FOR SUGARS

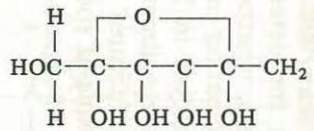
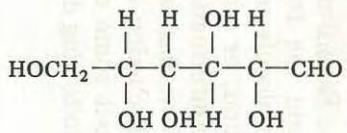
The following section gives selections from the Rules of Carbohydrate Nomenclature of the American Chemical Society (1963) and of the International Union of Pure and Applied Chemistry (1971). The former set is the result of study committees representing the American and the British Chemical Societies. Those rules selected are given in an order related to the structures variously used for the sugars as shown in Table 1.1, and which have special bearing upon the intrinsic chemistry of the sugars.

Aldose and Ketose Sugars

The use of the terms *aldose* and *ketose* indicates, in a generic sense, the chemical character of the reducing (normal-chain) form of a sugar. For an aldehydo sugar the carbon atom with the aldehyde function RHC=O is carbon atom number 1, and the following carbon atoms are numbered in sequence. For the cyclic (hemiacetal) form of a sugar, carbon atom number one becomes the anomeric car-

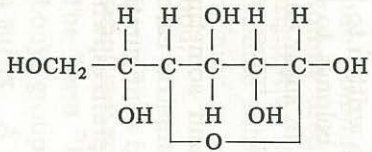
TABLE 1.1

DEVELOPMENT OF FORMULAS AND STRUCTURES FOR THE CONSTITUTION OF D-GLUCOSE

Level of Attainment	Formula or Structure	Investigator or School
Molecular Configuration		
Empirical formula. "Hydrate de carbone"; Kohlenhydrat; Carbohydrate	CHO, and then CH_2O when the structure of water was recognized to be H_2O	Dumas (1843)
Molecular composition based on $C_6H_{12}O_6$		Tollens (1883)
Normal chain aldehydo structure and proven molecular formula	$CH_2OH—(CHOH)_4CHO$ $C_6H_{12}O_6$	Kiliani (1886) Tollens, Mayer (1888)
Molecular configuration of normal chain structure		Fischer (1891)

Ring Structures

γ-Lactol form (furanoid)



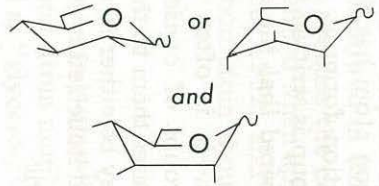
Fischer-Tollens formula (*Ca.* 1900)

Pyranoid

Hirst, Purves (1923)
Charlton *et al.* (1926)
Haworth (1928)

Ring Conformations

The "normal" chair, "alternate" chair, and boats



Haworth (1928)
Isbell (1937)
Hassel and Ottar (1947)
Reeves (1949)
Angyal (1969)

bon atom. For ketoses, the carbonyl atom $R-\overset{\overset{O}{\|}}{C}-R$ has the lowest possible number.

To indicate the total number of carbon atoms in a molecule, such as 3 to 7, the terms triose, tetrose, pentose, hexose, and heptose are used; thus there are aldotrioses and ketotrioses. The monose (formaldehyde) and the diose (acetaldehyde) are not usually considered to be sugars.

Configurational Relationships

Enantiomers.—Enantiomeric or mirror-image relationships between the sugars are indicated by the use of the small capital letters D and L. The significance of these symbols is discussed in detail in Chapter 2. Briefly, a sugar belongs to the D-family of sugars when its highest numbered asymmetric carbon atom has the OH group written to the right in the Fischer projection formula. The sugar belongs to the L-family when that OH group is written to the left. In sugar enantiomers it must be remembered that the configuration is transposed at all asymmetric centers.

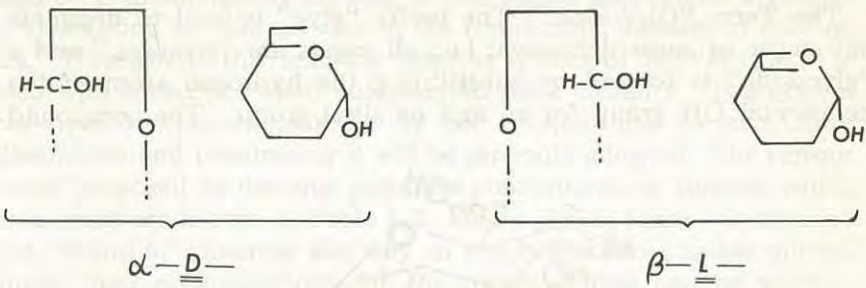
Configurational Prefixes.—It is often necessary, and sometimes convenient, to describe groups of *consecutive* asymmetric carbon atoms by a prefix which relates them to the established configuration of another sugar, which may be either D- or L-; these prefixes, which are always uncapitalized and italicized in print, are:

Number of Asymmetric Carbon Atoms	Prefix
1	*glycero*
2	*erythro*, *threo*
3	*arabino*, *lyxo*, *ribo*, *xylo*
4	*allo*, *altro*, *galacto*, *gluco*, *gulo*, *ido*, *manno*, *talo*

In usage the prefix precedes the term describing the number of carbon atoms in the chain.

Anomers.—The configurational symbols α- and β- indicate that the configuration of hydroxyl substituents or substituted hydroxyl substituents at the anomeric carbon atom (the asymmetric carbon atom created by the ring structure of the sugars) is the same as or the opposite of that for the enantiomeric or reference carbon atom. In the Fischer-Tollens projection formula, that OH group is written to the right for the α-D-anomer, and to the left for the β-D-anomer. The reverse is true for the L-series of sugars. Because the OH disposition about the anomeric carbon atom is the same for an α-D- and a β-L-

sugar, rules of carbohydrate nomenclature state that an anomeric specification must always be followed by an enantiomeric specification.



The preceding rule has been adopted by the International Nomenclature Committee and is followed in this text, but it has not been uniformly used in the past because the concept of "same" or "like" configuration is not well defined when applied to carbon atoms bearing unlike substituents (Eliel 1962) and because there is difference in opinion as to which carbon atom should be used as the reference carbon.

Descriptive Terms

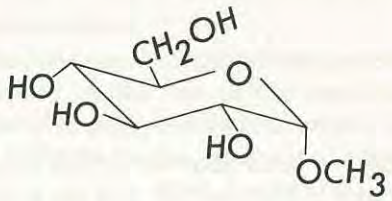
The Term "Ulose."—The term "-ulose" indicates a ketose with the carbonyl group at the carbon atom number 2 position, and it is used as a suffix. A prefix, such as "glycero-," denotes the number of carbon atoms in the chain and it, in turn, is preceded by a term designating the configuration of the group of asymmetric carbon atoms present. Certain trivial names established by usage are permitted. For example, using a configurational prefix, another name for D-fructose is D-*arabino*-hexulose. Ketoses having 2 ketonic carbonyl groups are named by means of the suffix "-diulose."

The Term "Deoxy."—When an OH group of a sugar is replaced by a hydrogen atom, a numerical prefix indicating the carbon atom, a hyphen, and the term "deoxy" precede the configurational description of the sugar. For example, the second carbon atom in 2-deoxy-D-glucose is a methylene ($-CH_2$) carbon atom. For 6-deoxy-D-galactose (D-fucose) and 6-deoxy-L-mannose (L-rhamnose) it is a methane carbon atom.

The Terms "Furanose" and "Pyranose."—The size of a sugar ring is indicated by following the root of the sugar name, such as *gluco*, *galacto*, and *manno* with the term "furanose" for a 5-membered heterocyclic ring, and "pyranose" and "septanose" for 6- and 7-mem-

bered heterocyclic rings, respectively. For glycosides, the size of the ring is indicated by inserting the term "furan" or "pyran" in the name. For example, derivatives of glucose may be either glucofuranosides or glucopyranosides.

The Term "Glycoside."—The prefix "glyc" is used to designate any sugar or sugar derivative; i.e., all sugars are "glycoses," and a "glycoside" is formed by substituting the hydrogen atom of the hemiacetal OH group for an aryl or alkyl group. The compound



is, generically, a methyl glycoside. Specifically, it is methyl α-D-glucopyranoside.

The Term "Oligosaccharide."—Oligosaccharides are relatively low molecular weight sugars which yield monosaccharides on hydrolysis. Nonreducing disaccharides are called glycosyl glycosides, and reducing disaccharides are called glycosyl glycoses. The nonreducing disaccharide sucrose can be described as either β-D-fructofuranosyl-α-D-glucopyranoside or α-D-glucopyranosyl-β-D-fructofuranoside. The need for describing the position of the glycosidic linkage between the monosaccharide units is obviated, since both anomeric OH groups participate in the formation of the joining bond, indicated by the suffix "-side." The reducing disaccharide α-D-lactose is 4-*O*-β-D-galactopyranosyl-α-D-glucopyranose. It can also be described as *O*-β-D-galactopyranosyl-(1 → 4)-α-D-glucopyranose. This method is employed to name higher molecular weight oligosaccharides; it describes the structure of the compounds precisely but it becomes progressively cumbersome to use in print and it is nearly impossible to use in spoken language. Therefore, trivial names such as stachyose and kestose may be preferred, but these have the disadvantage of not revealing molecular structure. For naming oligosaccharides, Bailey (1965) uses the Whelan method, which has many merits, and this also shortens the monosaccharide name by using the first three letters for the monosaccharide units and *p* and *f* for pyranose and furanose.

The Italic *O*.—The italic *O* in the name of a compound indicates that a hydrogen atom of a specific OH group has been replaced by some other substituent.

SPECIFICATION OF CONFORMATIONS

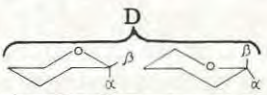
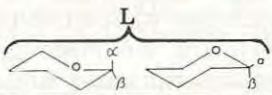
A standard method for naming the various furanose and pyranose conformations of the sugars has not yet become available. No one method is unambiguous for special conceptual approaches that arise in attempting to indicate various conformational isomers of the sugars. Throughout this text the original system of Reeves (1949) is used with subscripts when necessary to show chirality. This system has been tentatively adopted by the International Nomenclature Committee and presumably it will be generally adopted. The various terms proposed to describe pyranose conformational isomers which have merit are shown in Table 1.2. Mill's (1955) terms "*O*-outside" and "*O*-inside" describe the way in which the two possible mirror-image chair conformations for the pyranose ring can be written. Angyal's (1965) descriptive terms "normal" and "alternate," are convenient to use, particularly when the two chair conformations possible for a given enantiomer are being discussed.

The CA and CE specifications of Isbell and Tipson (1960) and those of Guthrie (1958) specify the disposition of an anomeric OH substituent and therefore utilize a secondary feature of ring conformation to specify the primary feature. CA and CE indicate whether the anomeric hydroxyl substituent is axial or equatorial, respectively, *for the α-anomer only*.

The proliferation of symbols to specify pyranose ring conformations has as its origin what seems to be a simple question, namely,

TABLE 1.2

SYMBOLS AND TERMS PROPOSED FOR DESIGNATING CHAIR CONFORMATIONS OF PYRANOID SUGARS

Reference				
	D		L	
Reeves (1949)	C1	1C	1C	C1
Mills (1955)	"O-outside"	"O-inside"	"O-inside"	"O-outside"
Isbell (1956)	C1	C2	C1	C2
Guthrie (1958)	α-(Ca)	α-(Ce)	α-(Ca)	α-(Ce)
Isbell, Tipson (1960)	CA	CE	CA	CE
Angyal (1965)	Normal	Alternate	Normal	Alternate
Current (1969)[1]	$C1_{(D)}$	$1C_{(D)}$	$C1_{(L)}$	$1C_{(L)}$
Szejtli (1969)	$^{4}C_{1}$	$^{1}C_{4}$	$^{1}C_{4}$	$^{4}C_{1}$

[1] See Carbohydrate Research starting with Vol. 1, 1966.

"Should enantiomeric sugars have the same, or a mirror-image conformational symbol?" Proponents of a system for identical symbols argue that enantiomers normally exist in the same conformation, and that the chiral specification D- or L- is adequate in itself. They also feel that since mirror-image symbols are used for the two possible chair conformations of one enantiomer, the use of different mirror-image symbols for the favored chair conformations of the other enantiomer, while self-consistent, is not desirable.

The second school takes the position that, because the enantiomeric sugars in their favored conformation do not have the same steric arrangement of the atoms in the ring and one ring structure is actually the mirror-image structure of the other, this fact should be acknowledged by the use of mirror-image symbols. This is essentially the original system of Reeves. One attribute is that, as one systematically considers the nomenclature of the sugars, the concepts of configuration and conformation are separate, and a configuration alteration does not affect a conformational specification, and vice versa. This is not the case when identical symbols are used to describe the favored chair structure of enantiomers.

A single enantiomer can exist in two chair conformations, and the carbon-oxygen ring in one case is the mirror image of the shape of the ring in the other case. The configuration of the substituents at each carbon atom is not altered in transforming from one chair structure to the other, but the spatial position of the substituents with respect to that of the ring is altered. Hence Reeves (1949) assigned mirror-image conformational symbols to the ring shapes of a single enantiomer to acknowledge this fact also.

Symbols used to the greatest extent currently are those of Reeves with the addition of a chiral designation such as $^{C1}_{D}$ when the chirality of the compound has not been previously indicated. Unless the chirality of the compound is known, the C1 and 1C conformational symbols are meaningless (Durette and Horton 1971). Numerical superscripts and subscripts accompanying a chair designation are illustrative (Szejtli 1969), but cumbersome. Symbols of this order are used, however, to designate furanose ring conformations, as shown in the next chapter.

BIBLIOGRAPHY

AMERICAN CHEMICAL SOCIETY. 1963. Rules of carbohydrate nomenclature. J. Org. Chem. *23*, 281-291.

ANGYAL, S. J. 1965. Conformational analysis in carbohydrate chemistry. *In* Conformational Analysis, E. Eliel *et al.* (Editors). Interscience Publishers, New York.

ANGYAL, S. J. 1969. The composition and conformation of sugars in solution. Angew. Chem. (International Edition) *8*, 157-166.

ARMSTRONG, E. F. 1903. Studies on enzyme action. I. The correlation of the stereoisomeric α- and β-glucosides with the corresponding glucoses. J. Chem. Soc. *83*, 1305-1313.

BAEYER, A. 1870. On dehydration and its importance to the plant kingdom and fermentation. Ber. deut. chem. Ges. *3*, 63-75.

BAILEY, R. W. 1965. Oligosaccharides. The Macmillan Co., New York.

BÖESEKEN, J. 1913. On the arrangement of hydroxyl groups of polyhydroxy compounds in space. The configuration of the saturated glycols and of α- and β-glucose. Ber. deut. chem. Ges. *46*, 2612-2628.

BROWN, H. C., BREWSTER, J. H., and SCHECTER, H. 1953. An interpretation of the chemical behavior of five- and six-membered ring compounds. J. Am. Chem. Soc. *76*, 467-474.

CHARLETON, W., HAWORTH, W. N., and PEAT, S. 1926. A revision of the structural formula for glucose. J. Chem. Soc. 89-101.

DREW, H. D. K., and HAWORTH, W. N. 1926. A critical study of ring structure in the sugar group. J. Chem. Soc. II. 2303-2310.

DUMAS, J. B. 1843. Traité de Chimie *6*, 273.

DURETTE, P. L., and HORTON, D. 1971. Conformational analysis of sugars and their derivatives. Advances in Carbohydrate Chem. and Biochem. *26*, 49-125.

ELIEL, E. L. 1962. Stereochemistry of Carbon Compounds. McGraw-Hill Book Co., New York.

FISCHER, E. 1891. On the configuration of grape sugar and its isomers. Ber. deut. chem. Ges. *24*, 1836-1845.

FITTIG, R. 1871. On the Constitution of the so-called Carbohydrates. Tubingen.

GUTHRIE, R. D. 1958. A nomenclature for sugar conformers. Chem. Ind. (London) 1593-1594.

HASSEL, O., and OTTAR, E. 1947. The structure of molecules containing cyclohexane or pyranose rings. Acta Chem. Scand. *1*, 929-942.

HAWORTH, W. N. 1928. Constitution of the Sugars. Edward Arnold and Co., London.

HIRST, E. L., and PURVES, C. B. 1923. The structure of the normal monosaccharides. I. Xylose. J. Chem. Soc. *123*, 1352-1360.

HUDSON, C. S. 1909. The significance of certain numerical relations in the sugar group. J. Am. Chem. Soc. *31*, 66-86.

HUDSON, C. S. 1941. Emil Fischer's discovery of the configuration of glucose. J. Chem. Educ. *18*, 353-357.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY. 1969. Tentative rules for carbohydrate nomenclature. Part I. 1971. Biochemistry *10*, 3883-4004.

ISBELL, H. S. 1937. Configuration of the pyranoses in relation to their properties and nomenclature. J. Res. Nat. Bur. Std. *18*, 505-534.

ISBELL, H. S. 1956. System for classification of structurally related carbohydrates. J. Res. Nat. Bur. Std. *57*, 171-178.

ISBELL, H. S., and TIPSON, R. S. 1960. Conformations of the pyranoid sugars. I. Classification of conformers. J. Res. Nat. Bur. Std. *64A*, 171-176.

KILIANI, H. 1886. On the effect of hydrogen cyanide on dextrose. Ber. deut. chem. Ges. *19*, 767-772.

MILLS, J. A. 1955. Cyclic derivatives of carbohydrates. Advances in Carbohydrate Chem. *10*, 1-53.

REEVES, R. E. 1949. Cuprammonium-glycoside complexes. V. The conformation of the pyranose ring in some D-hexose anhydrides. J. Am. Chem. Soc. *71*, 2116-2119.

SMITH, J. H. C. 1949. Products of photosynthesis. *In* Photosynthesis in Plants, J. Frank and W. E. Loomis, Editors. The Iowa State College Press, Ames.

SZEJTLI, J. 1969. A new formula for the description of the conformation of pyranoid sugars. Acta Chim. Acad. Sci. Hung. *61*, 57-68.

TANRET, C. 1895. On the modifications of molecules of glucose. Compt. rend. *120*, 1060-1062.

TOLLENS, B. 1883. On the behavior of dextrose to ammoniacal silver solutions. Ber. deut. chem. Ges. *16*, 921-924.

TOLLENS, B., and MAYER, F. 1888. On the determination of the molecular weight of raffinose and of the formaldehydes using Raoult's freezing method. Ber. deut. chem. Ges. *21*, 1566-1572.

Structure of Monosaccharides

The monosaccharides are the simplest form of the sugars that still retain the identity of the sugars, such as nonvolatility and sweet taste. Oligosaccharides are compounds containing several (2 to perhaps 10) monosaccharide units. They are formed by the elimination of a molecule of water between two OH groups to form an intramolecular glycosidic bond. They can be hydrolyzed to generate the component monosaccharides. The composition and properties of the oligosaccharides are presented in Chapter 3.

TYPES OF ISOMERISM

Many naturally occurring compounds have the same molecular formulas, such as $C_6H_{12}O_6$, but differ in chemical or physical properties. They are said to be isomers (Gr. *isos*, equal). There are two fundamental isomeric types, *structural* and *spatial*. Two compounds that are isomers can be either structural isomers or stereoisomers (Gr. *stereos*, three-dimensional, spatial), but never both. Structural isomers may be carbon chain isomers that have the same molecular formula, but one has a normal and the other a branched chain. They may also be functional group isomers, such as compounds with the same molecular formula, but one is an aldehyde and the other a ketone. Among the monosaccharides, ring forms of different size are also encountered. These structural isomers are easily interconvertible forms, or tautomers (Gr. *tautos*, the same).

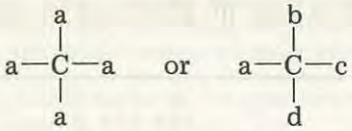
Stereoisomers differ in the arrangement of their atoms in space. Two types of stereoisomerism are *configurational* and *conformational* isomerism. They are of fundamental importance to the structure and the intrinsic chemistry of the sugars in foods and must be given detailed attention and definition.

Aldose Configurational Isomers

Configuration describes a molecule in space by not only stating the number of covalent bonds by which each atom is joined to other atoms, but also by specifying the geometrical arrangement of all the atoms (Wheland 1960). The spatial disposition of the 4 different atoms or groups of atoms with which the tetrahedral carbon atom can combine is the simplest case (Eliel 1962).

Tetrahedral Nature of the Carbon Atom.—The carbon atom is tetravalent and can combine with 4 identical or 4 entirely different

substituents or groups of substituents, such as:



Compound Ca,a,a,a is symmetrical and can exist only in one form, but compound Ca,b,c,d is not symmetrical and can exist in two configurational forms, one being the exact nonsuperposable mirror image of the other.

The existence of compounds whose structures are alike in all respects except that one is a mirror-image of the other, was first recognized with tartaric acid by Louis Pasteur. The existence of the two compounds intrigued Pasteur, and although the theory of the tetrahedral nature of the carbon atom was not known at the time, Pasteur (1860) asked:

> Are the atoms of the *dextro* acid grouped on the spirals of a dextrogyrate helix, or placed at the summit of an irregular tetrahedron? They (the two forms of tartaric acid) resemble each other as the right hand resembles the left hand, or better, as two irregular but symmetrical tetrahedra; and these analogies and differences remain in all their derivatives.

In 1874 van't Hoff and Le Bel (cf Larder 1967) independently proposed that the geometry of the four valences of the carbon atom is such that each valence projects toward the apex of a regular (symmetrical) tetrahedron, and that when four *different* groups are combined with the carbon atom, a potential for dissymmetry is generated which results in the asymmetric carbon atom. A carbon atom enclosed in a transparent regular tetrahedron with each valence directed toward an apex of the tetrahedron is shown in Fig. 2.1.

The term "dissymmetric" is used to indicate degraded symmetry, such as that generated by placing four unlike substituents at the apices of a regular and symmetrical tetrahedron. It is possible only in forms which do not possess a plane or center of symmetry (Lowry 1935). Eliel (1962) pointed out that a dissymmetric molecule lacks an alternating axis of symmetry, and is usually optically active, but not necessarily so. A dissymmetric molecule may or may not be asymmetric, i.e., lacking a simple axis of symmetry.

Asymmetry is used, through reference to chiral (Gr. *cheir*, hand) properties to designate right- or left-handed properties of an object. Of the terms "chiral" and "asymmetric," which are related, "chiral" is more formal. There are centers, axes, and planes of chirality which lead to asymmetry (Bentley 1969).

With the introduction of the topic of chirality, which relates the

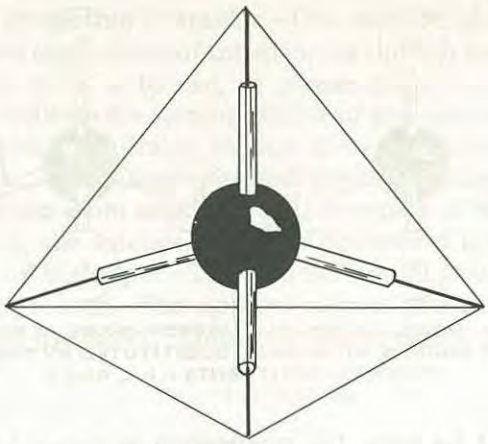


FIG. 2.1. TETRAHEDRAL DIRECTION OF THE 4 VALENCES OF THE CARBON ATOM

geometry of an object to "handedness," care must be taken not to confuse a *specification* of chirality, such as *dextro* or *levo*, with the actual handedness of the object. The terms "dextro" and "levo" are used in two ways. In the first, reference is made to the direction in which a plane of polarized light is bent by an optically active object; e.g., a compound may be either dextrorotatory, or levorotatory, or neither. By convention, a dextrorotatory compound will bend a beam of plane-polarized light to the right as an observer views it in the eyepiece of a polarimeter, but this cannot be caused by an object that is geometrically right-handed. It must be left-handed instead. Pasteur's question, "Are the atoms of the dextro acid grouped on the spirals of a dextrogyrate helix?", apparently refers to the early convention which specified the direction as viewed *along the beam of light*. This matter is discussed further in Chapter 3. In the second sense, *dextro* and *levo* refer to the configuration about an asymmetric carbon atom. This subject is discussed next.

Asymmetric Carbon Atoms.—A carbon atom with four unlike atoms or groups at the apices of a symmetrical tetrahedron does not possess any element of symmetry because it has a center of chirality which immediately leads to the possible existence of mirror-image forms. One is a right-handed structure and the other is a left-handed structure. To establish whether an asymmetric carbon atom *Cabcd* is right- or left-handed requires the convention that the four substituents be assigned a priority sequence such as $a > b > c > d$. These four hypothetical substituents are placed at the valences of the tetrahedral carbon atoms shown in Fig. 2.3. If the two carbon atoms are viewed so that the substituent d, which has the lowest

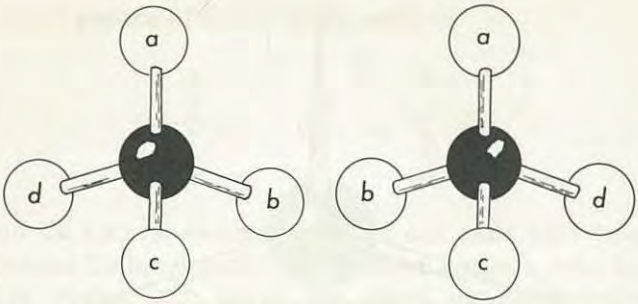
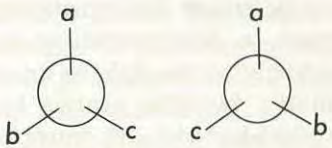


FIG. 2.2. NONSUPERPOSABLE MIRROR-IMAGE STRUCTURE OF THE CARBON ATOM WHEN SUBSTITUTED BY THE 4 DIFFERENT SUBSTITUENTS a, b, c, AND d

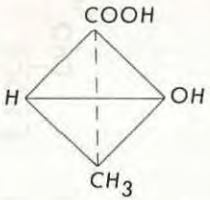
priority, cannot be seen, the progression sequence for *abc* is clockwise or right-handed in one case, and counterclockwise or left-handed in the other. These four hypothetical substituents placed at the valences of the tetrahedral carbon atoms shown in Fig. 2.2 then create mirror-image compounds. If the two carbon atoms are viewed so that the substituent *d*, which has the lowest priority, cannot be seen, the progression sequence for *abc* is clockwise or right-handed in one case, and counterclockwise or left-handed in the other.



No matter how the two carbon atoms are oriented, one cannot be superposed upon the other. The concept of nonsuperposability is of course abstract, since no object which occupies space can be "superposed" upon another.

A discussion of sequence rules and the use of configurational labels R and S is given by Bentley (1969). When applied to the sugars, certain carbon atoms that are *dextro* (or *rectus*, R) in one formula become *levo* (or *sinister*, S) in another. This is an unfortunate consequence of the need to specify configuration by relating it to the nature of the asymmetric carbon atom substituents. In the Fischer projection formula for D-glucose, carbon atom number 4 is R, but in the pyranose structure, it is S. The use of the configurational labels R for *rectus* (Gr., right) and S for *sinister* (Gr., left) is now universally used to avoid the two-fold meaning of *dextro* and *levo*, and the latter are used to denote optical rotation only.

Fischer Projection Formulas.—One method of specifying the three-dimensional disposition of substituents and the tetrahedral features of the carbon atom is to use, in planar form, the apices of the tetrahedron to indicate the stereoposition of the carbon atom substituents. The tetrahedron is drawn so that there is an unseen edge, drawn as a dotted line. All 4 substituents are depicted as being visible. Visualizing the carbon atom as occupying the center of the three-dimensional tetrahedron, the valence projected *downward* is perceived as being in the plane of the paper. The two horizontal valences project *forward* toward the reader. The *upward* valence projects backward through the plane of the paper. Thus, D-(-)-lactic acid is often shown as

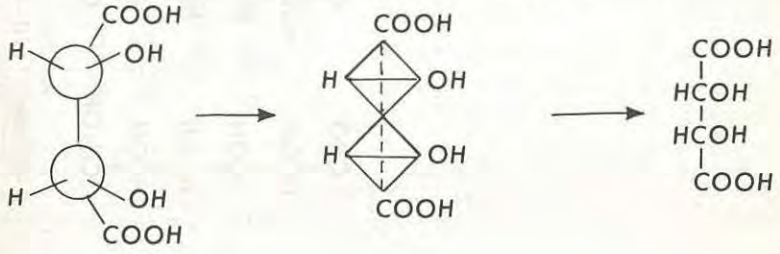


(-) - LACTIC ACID

with the C—CH_3 bond in the plane of the paper, the C—H and C—OH bonds projected toward the reader, and the C—COOH bond projected backward through the plane of the paper.

This method of planar notation of the tetrahedral carbon atom forms the basis of the Fischer projection formulas, and while they are very useful, failure to apply the restrictions placed on the formulas through application of the conventions they contain may lead to misunderstanding.

In Fischer projection formulas, *all tetrahedral apices which connect carbon atoms lie in the plane of the paper* on which they are written. To do this requires that the model be turned as the projection is made, and each asymmetric carbon atom be viewed individually. The results are then recorded vertically. The projection formula for *meso*-tartaric acid generated from the planar tetrahedral formula which, in turn, was generated from a perspective model is:



CHO
HCOH
HCOH
HCOH
HCOH
CH2OH
D-Allose
CHO
HOCH
HCOH
HCOH
HCOH
CH2OH
D-Altrose
CHO
HCOH
HOCH
HCOH
HCOH
CH2OH
D-Glucose
CHO
HCOH
HOCH
HOCH
HCOH
CH2OH
D-Mannose
CHO
HCOH
HCOH
HOCH
HCOH
CH2OH
D-Gulose
CHO
HOCH
HCOH
HCOH
HCOH
CH2OH
D-Idose
CHO
HCOH
HOCH
HOCH
HCOH
CH2OH
D-Galactose
CHO
HOCH
HOCH
HOCH
HCOH
CH2OH
D-Talose

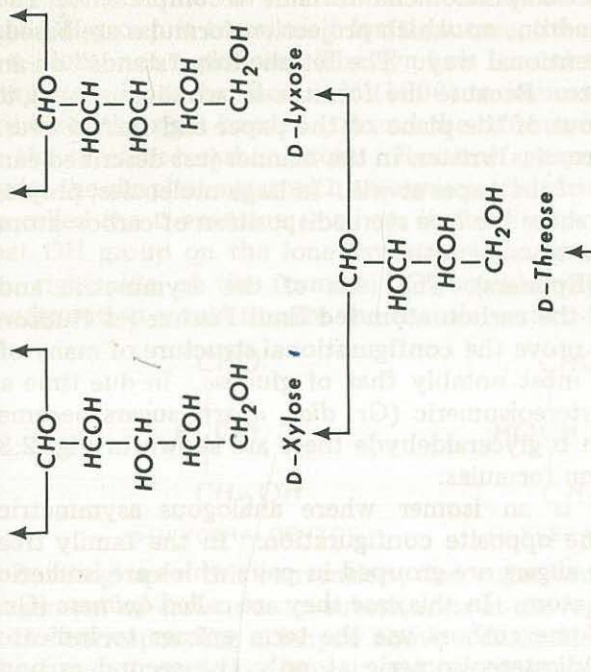


FIG. 2.3. FAMILY TREE OF D-ALDEHYDO SUGARS

Obviously, projection formulas do not show the direction of the four valences of the asymmetric carbon atom and this makes sterically directed reaction phenomena difficult to comprehend. The drawing of the tetrahedron, on which projection formulas are based, is not done in a conventional way. The tetrahedron "stands" on an apex rather than a face. Because the formula is two-dimensional, it may never be lifted out of the plane of the paper and turned over. Fischer projection formulas written in the manner just described can be turned in the plane of the paper at will. In large molecules, projection formulas do not show the true stereodisposition of carbon atom substituents in relation to one another.

Diastereoisomers (Epimers).—The idea of the asymmetric and tetrahedral nature of the carbon atom led Emil Fischer (cf Hudson 1941) to deduce and prove the configurational structure of many of the aldohexoses, but most notably that of glucose. In due time a "family tree" of diastereoisomeric (Gr. *dias*, apart) sugars became known. Starting with D-glyceraldehyde these are shown in Fig. 2.3 using Fischer projection formulas.

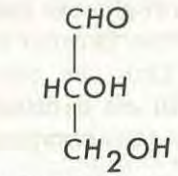
A *diastereoisomer* is an isomer where analogous asymmetric carbon atoms have the opposite configuration. In the family tree shown in Fig. 2.3 the sugars are grouped in pairs which are isomeric at the second carbon atom. In this case they are called *epimers* (Gr. *epi*, above, over). Some authors use the term epimer to indicate compounds that are diastereoisomeric at only the second carbon atom. Others use the term for other asymmetric carbon atoms. In the latter sense, D-glucose and D-galactose are then 4-epimers.

Enantiomers.—Enantiomers (Gr. *enantio-*, opposite, opposed) are stereoisomers which bear a total mirror-image relation to each other, and are entirely nonsuperposable. They are generated by transposing the configuration of the carbon atom substituents at all of the asymmetric carbon atoms of the molecule.

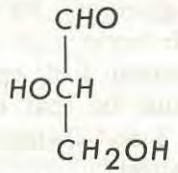
For a compound containing n asymmetric carbon atoms there are 2^n diastereoisomers possible. For an aldehydo hexose with 4 asymmetric carbon atoms in the acyclic form, 2^4 configurational isomers are possible. For a given aldohexose, progressively transposing the configuration about each asymmetric center generates distinct diastereoisomers until finally, when all transpositions have been made, the complete mirror-image structure of the starting aldohexose is obtained. For a given aldohexose, 2^3 diastereoisomers are possible, and when there is transposition of configuration at the *reference* carbon atom, each of the 8 diastereoisomers generates an enantiomer, to yield a total of 16 aldehydo hexose configurational isomers.

While the fifth carbon atom of the aldehydo hexoses is the

reference carbon atom, and transposing the configuration at this atom alone generates a sugar belonging to another chiral family, it does not generate an enantiomer of the same sugar. If, in Fig. 2.3 that OH group is transposed for D-glucose, the compound generated is L-idose, not L-glucose. The symbols D- and L- (cf Hudson 1948) were introduced by Rosanoff (1906) to indicate which enantiomer possesses the right-handed (D) and the left-handed (L) configuration at the *reference* carbon atom. Rosanoff proposed that glyceraldehyde, the simplest sugar with an asymmetric carbon atom (glycerose) be called the D-enantiomer when, in the Fischer projection formula, that OH group on the lone asymmetric carbon atom is written on the right side of the formula. Glyceraldehyde with the opposite configuration would then be the enantiomeric L-form.



D-GLYCERALDEHYDE



L-GLYCERALDEHYDE

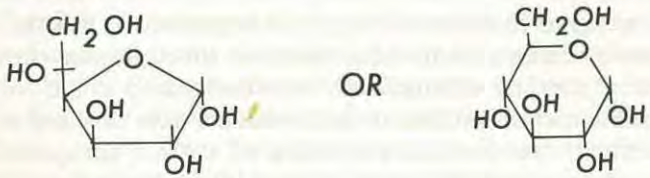
Building upon this convention, that sugar which has the same arrangement of carbon atom substituents at the *reference* carbon atom as the corresponding aldehyde above is designated as a D- or L-sugar. These enantiomeric configurational symbols have nothing to do with the direction toward which a solution of a given compound rotates the plane of polarized light when viewed in a polarimeter. The use of symbols such as (+) and (−) in the name of a compound, however, does indicate the direction that a solution of a compound rotates polarized light; but this is a physical property, not a structural designation; *d* for *dextro* (Lat. *dexter*, right) or *l* for *levo* (Lat. *laevus*, left) were formerly used to indicate that the light is rotated to the right or left, respectively.

D-glyceraldehyde rotates the plane of polarized light to the right and can be described as D-(+)-glyceraldehyde, but (-)-lactic acid, as shown on page 21, has the D-configuration at the reference carbon atom, and is therefore D-(-)-lactic acid. The direction toward which a solution of a compound will rotate polarized light cannot be simply related to the configuration of the reference carbon atom for several reasons. In the case of lactic acid, the compound forms a dimer in solution that has a negative rotation. The glyceraldehydes exhibit mutarotation, which is best explained by dimer formation (Baer and Fischer 1939); the L-amino acids, which have the L-glyceraldehyde configuration, are generally dextrorotatory. One important naturally

occurring sugar, D-fructose, has a negative rotation because its favored ring structure (conformation) has an overall element of levorotatory power.

Anomers.—The final example of configurational isomerism for the sugars is the existence of "upper" epimers or *anomers* (Gr. *anos*, upper). These diastereoisomers have the opposite configuration at a special asymmetric carbon atom arising from cyclization of the aldehyde or keto group.

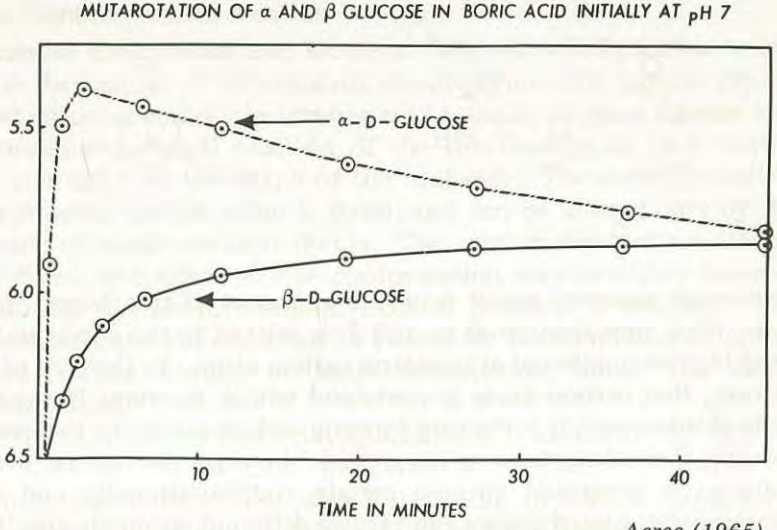
The presently accepted configurational assignment for the anomers of D-glucose stems mainly from the studies of Böeseken (1913). Recognizing that *cis* vicinal glycols react with boric acid to enrich the acidity of their solutions, Böeseken monitored the change in conductance of solutions of boric acid containing freshly dissolved α-D- and β-D-glucose. He found that α-D-glucose reacted instantaneously with boric acid while β-D-glucose became reactive only after mutarotation had commenced. Thus, he concluded that α-D-glucose must be that compound with *cis* hydroxyl groups at carbon atoms 1 and 2, and β-D-glucose is that compound with the *trans* configuration.



Böeseken's results have been repeated by others (MacPherson and Percival 1937; Shallenberger *et al.* 1965). The reaction, as monitored by changing pH (Acree 1965) is shown in Fig. 2.4.

This configurational assignment comes up for periodic challenge for a variety of reasons. Perhaps foremost among them is the fact that the glycol-borate reaction is very complex (Acree 1973), and its products are weakly ionized esters that can exist in several forms. As such they are very useful in the study of the structure of various polyhydroxy compounds (Gorin and Mazurek 1973).

One of the criticisms of Böeseken's results is that they seem to require the glucofuranose ring structure (Sickles and Schultz 1964), which is not the favored form, and has never been observed in water solution. Such criticism is not valid, since it equates principles of sugar structure derived from "resting" molecules with those of a reacting molecule. Mazurek and Perlin (1963), using nuclear magnetic resonance spectroscopy, found strong evidence that it actually is the furanose structure of the α-anomer that forms the 1,2 cyclic

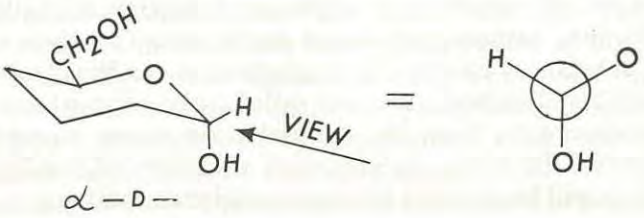


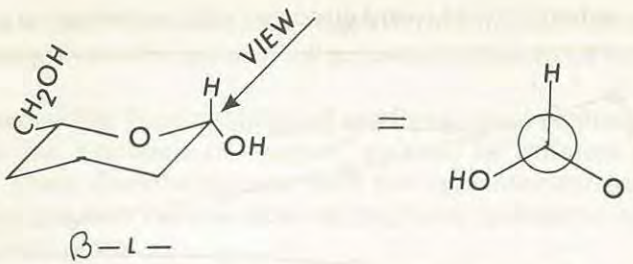
Acree (1965)

FIG. 2.4. REACTION OF α-D- AND β-D-GLUCOPYRANOSE WITH BORIC ACID AS MONITORED BY CHANGING pH

borate ester. In the pyranose form, α-D-glucose may react with borate by virtue of a boat conformation (B3). In any event, Böeseken's findings and deductions hold, and whether or not the reactive α-D-glucose form is a furanose or pyranose ring, or both, is not pertinent to the argument.

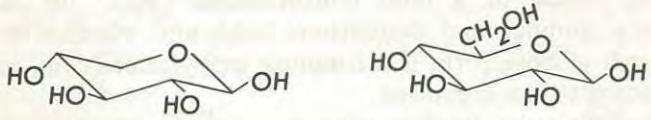
The fact that the configuration about the anomeric carbon atom is the same for α-D- and β-L-sugars is quite apparent in conformational and Newman (1955) projection formulas. In the latter formulas, configuration is viewed by looking along the axis of the C_1 to C_2 bond, and the C_1 substituents can be numbered in clockwise direction. In a total conformational sense, however, the substituents do *not* have the same stereodisposition with respect to the ring. In the β-anomers, in the C1 conformation, whether D- or L-, the anomeric hydroxyl group lies close to the average plane described by the entire ring. In the α-hexose anomers, it is perpendicular to the average plane of the ring.





β—L—

Although anomers result from the existence of ring forms of the sugars, their specification as α- and β- is related to the configuration of the highest-numbered asymmetric carbon atom. In the case of the pentoses, that carbon atom is contained within the ring. In the case of the aldohexoses, it is the ring-forming carbon atom. In the case of heptoses, the reference atom lies outside the ring. As a consequence, confusion is generated because certain configurationally and conformationally related sugars can receive different anomeric specification, such as α-L-arabinose and β-D-galactose. According to the Reeves' system, the symbol for designating conformation also depends on the configuration of the highest-numbered asymmetric carbon atom.



α-L-ARABINOSE β-D-GALACTOSE

Isbell and Pigman (1937) suggested that the naming of anomers should be related to the geometry of the ring to avoid this problem. They suggested that the names of members of an α- and β-pair of sugars ought to be selected so that when the oxygen atom lies to the right in the Fischer-Tollens projection formula, the more dextrorotatory member of the pair is designated α-. When the oxygen atom lies to the left, the less levorotatory member is then β-. Another way of stating this idea is that substances which have like configuration for the glycosidic and the ring-forming carbon atoms are called α-, while compounds which have unlike configuration are called β-. If the ring-forming carbon atom is not asymmetric (pentoses and ketohexoses), substances having like configuration for the glycosidic and highest-numbered carbon atom are called α-, and those having unlike configuration are β-; thus, the α-L-arabinose shown above becomes β-L-arabinose. In time, perhaps such problems of configurational specification will be resolved by conformational notation.

Aldose Conformational Isomers

Pyranose Conformational Isomers.—Whereas configuration spells out the disposition of substituents about asymmetric carbon atoms, conformation specifies the shapes that a molecule may assume and the consequent spatial position of the substituents of each carbon atom as a result of the shape of the molecule. The configuration of an asymmetric carbon atom is fixed, and can be altered only by the severence of single covalent bonds. The conformation of a molecule is not fixed, and although one conformation may be highly favored, the number of conformational isomers possible is infinite. The conformation of a molecule can be altered by the simple expedient of rotating carbon atoms, or their substituents, about the single covalent bonds.

The bond angle between contiguous carbon atoms linked through single covalent bonds in a molecule is 109°28′. Therefore, a 6-membered or 5-membered carbon atom ring, in order to be without strain, must assume one or more of a variety of possible unstrained forms. This is possible with conformational isomers.

The idea of conformational isomers for 6-membered rings had its origin in the proposal by Sachse (1890) that cyclohexane can exist either as a relatively rigid but strainless chair-like structure, or as a rather flexible, but also strainless boat-like structure. Drawn in perspective, these two basic conformational isomers of cyclohexane are:

CYCLOHEXANE

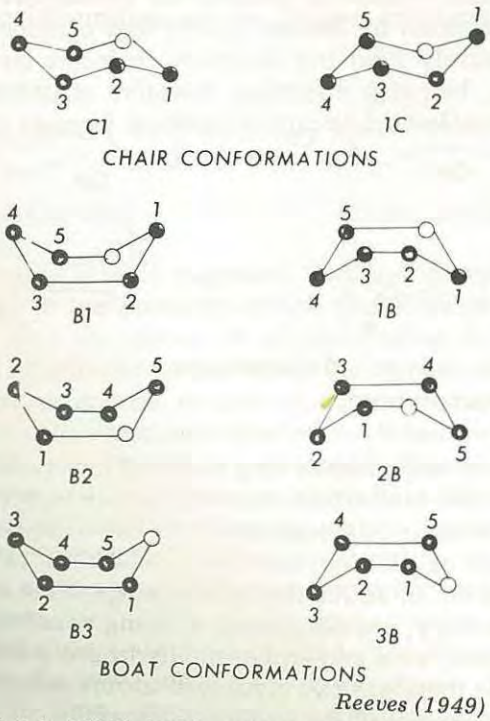
CHAIR CONFORMATION BOAT CONFORMATION

On viewing the cyclohexane ring in its various possible conformations it is clear that the carbon atom ring proton substituents are not identical. In the chair conformation 6 of them are perpendicular to the average plane of the ring, and are termed *axial* (a). The other 6 hydrogen atoms are in about the same average plane of the ring and, by terrestrial analogy, are designated as being *equatorial* (e). In the boat conformation, axial and equatorial hydrogen atoms again occur, and in addition, there are two hydrogen atoms called *bowsprits* (b) and two called *flagpoles* (f) by nautical analogy.

Although the C—O—C bond angle in the pyranose ring (104°) is

smaller than the C—C bond angle, the pyranose ring can assume chair and boat forms analogous to those described for the cyclohexane ring. The pyranose ring, however, may be more flexible than the cyclohexane ring (Brimacombe *et al.* 1958). The term conformation was introduced to organic chemistry when Haworth (1928) recognized that the pyranose ring could possess a number of different shapes, and that this would lead to a large field for inquiry. At the present time there is great emphasis on the conformational structure of the sugars, and many of their known chemical and biological properties are coming to be better understood through application of conformational principles, particularly their function in foods.

For practical purposes, there is only one chair form for cyclohexane, but two chair conformations are possible for the pyranose ring. Hassel and Ottar (1947) first pointed this out, and laid the foundation for deciding which of the pyranose chair conformations of a sugar is favored. Reeves (1949) codified and described the basic chair and boat conformations for the pyranose ring alone. These are shown in Fig. 2.5.



Reeves (1949)

FIG. 2.5. BASIC CHAIR AND BOAT CONFORMATIONS POSSIBLE FOR THE PYRANOSE RING

The two chair conformations are related to each other as an object is related to its mirror image and, because the carbon atoms are numbered, the structures are not superposable. If the carbon atoms were not numbered, the two chair structures shown would be symmetrical and therefore superposable. The carbon atoms are "labeled" for the sugars in the sense that the OH substituents on each carbon atom are not chemically equivalent.

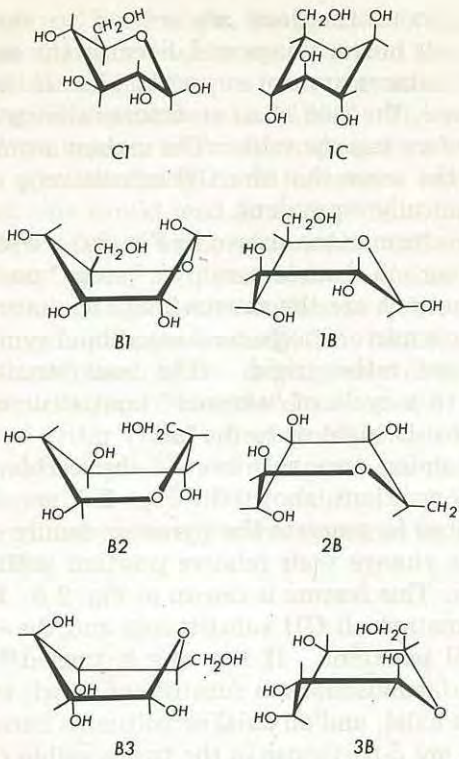
The six boat conformations shown in Fig. 2.5 are identified by the lowest-numbered carbon atom to occupy a "stem" position. Three of the boat conformations are the mirror-image structures of the other three, and they have mirror-image conformational symbols. Pyranose chair structures are rather rigid. The boat structures are quite flexible and lead to a cycle of "skewed" boat structures which may be skewed either to the right or to the left.

When the remaining two valences of the carbon atoms of the classic ring conformations shown in Fig. 2.5 are filled with the substituents required to generate the pyranose family of aldohexoses, those substituents change their relative position with respect to the ring conformation. This feature is shown in Fig. 2.6. For β-D-glucose in the C1 conformation all OH substituents and the $-CH_2OH$ group occupy equatorial positions. If the ring is turned "inside-out" to create the 1C conformation, each substituent which was equatorially disposed becomes axial, and all axial substituents become equatorial. Both compounds are β-D-glucose in the two possible chair conformations. The basic ring structures bear a mirror-image relation to each other, but the configuration of each individual carbon atom has not been altered.

In transposing a given boat structure to that of the alternate conformation, axial and equatorial substituents transpose, as well as the flagpole and bowsprit substituents.

One of the first principles of conformational analysis is that any bulky carbon atom substituent which occupies an axial position on a 6-membered ring imparts an element of instability to the compound. Thus, among the aldopyranoses, β-D-glucopyranose in the C1 conformation is the most highly favored structure, and the 1C conformation is the most unfavored structure. Because of their flexibility, boat forms are not as favored as either of the chair conformations (Durette and Horton 1971). However, the boat forms may be the reactive intermediates in many sugar reactions.

The C1 conformation for the D-series of aldohexopyranose sugars is shown in Fig. 2.7. The C1 conformations for both the D- and the L-series of aldopentopyranose sugars are also shown. The structures for the aldohexoses can be remembered, or constructed from memory,



Shallenberger and Acree (1971)

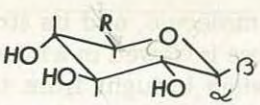
FIG. 2.6. BASIC CHAIR AND BOAT CONFORMATIONS FOR β-D-GLUCOPYRANOSE

Reproduced with permission of Springer-Verlag.

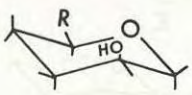
by noting that the key for the axial positions of the OH groups is 0, 2, 3, 4, 2–3, 3–4, 2–4, and 2, 3, 4 in reading the figure from glucose through idose. The aid for remembering the corresponding sugar names, which seems appropriate enough to one of their properties, is "A *G*ood *Ma*ny or *All Ga*llant *Al*terations *Gut* the *Ta*ste *Id*ea."

Those sugars with larger numbers of axial substituents can be expected to be in equilibrium, in solution, with substantial amounts of the alternate conformation. Examples are α-D-altrose, α-D-idose, and α- and β-D-ribose (Angyal 1969). It must be emphasized that the conformations shown for the sugars in Fig. 2.7 are not in all cases necessarily the favored conformation. For example, the favored conformation for α-D-arabinose is 1C rather than C1, and this is probably the reason why this D-sugar is levorotatory.

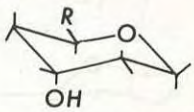
Construction of diagrams for the usually favored L-series of the aldohexopyranoses shown in Fig. 2.7 requires that an imaginary mir-



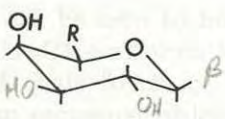
D – GLUCOSE
D – Xylose



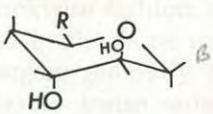
D – MANNOSE
D – Lyxose



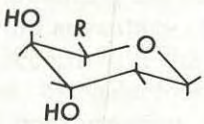
D – ALLOSE
D – Ribose



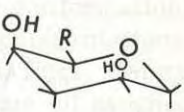
D – GALACTOSE
L – Arabinose



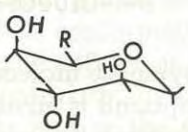
D – ALTROSE
D – Arabinose



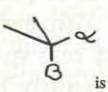
D – GULOSE
L – Lyxose



D – TALOSE
L – Ribose



D – IDOSE
L – Xylose



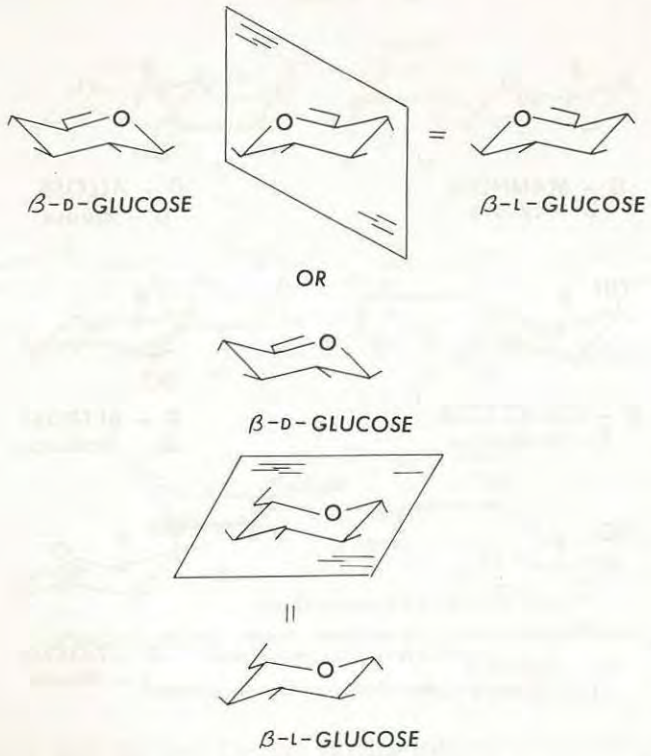
is the anomeric OH disposition for the L-series of sugars. R=H for Pentoses (lower case names) and $-CH_2OH$ for hexoses (capitalized names). OH groups not shown occupy an equatorial position.

Shallenberger and Acree (1971)

FIG. 2.7. C1 CONFORMATIONS FOR D-SERIES OF ALDOHEXOPYRANOSE AND ALDOPENTOPYRANOSES, AND ALSO L-SERIES OF ALDOPENTOPYRANOSES

Reproduced with permission of Springer-Verlag.

ror be placed before the molecule, and its structure drawn from the mirror. If β-D-glucopyranose is viewed in a mirror as shown below, the mirror-image structure, when brought from the mirror, is the structure of the nonsuperposable enantiomer β-L-glucopyranose.



In one case, the β-D-glucopyranose molecule is placed before a mirror; in the other case the compound is envisaged as being placed over a mirror. The images are identical, however, since lifting one structure from the plane of the paper and turning it over is permissible with perspective formulas. Operations permissible with various formulas are discussed in detail later in this chapter, but one special attribute of a ring conformational formula is that it can readily be determined whether or not a structure is C1 or 1C in either the D- or L-series.

To determine whether a ring structure has the 1C or C1 conformation, visualize, as shown in Fig. 2.8, a plane bisecting the single covalent bonds of the pyranose ring. For the $C1_{(D)}$ conformation, the ring oxygen atom and carbon atoms number 2 and 4 lie "above" the plane, and carbon atoms 1, 3, and 5 lie "below" the plane. If the ring is viewed from the side of the plane from which the oxygen atom

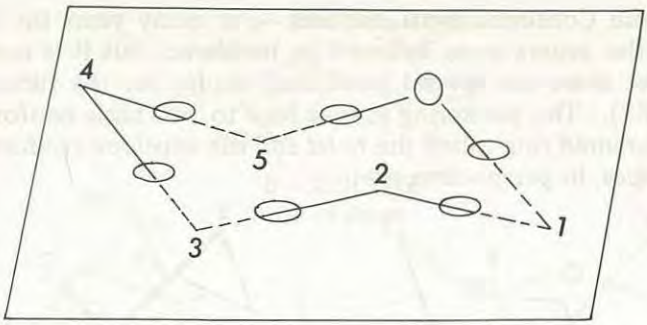


FIG. 2.8. IMAGINARY PLANE BISECTING THE SINGLE COVALENT BONDS OF A PYRANOSE C1 CONFORMATION

projects, it can be seen to be numbered in clockwise fashion, and is, therefore, the C1 conformation. When viewed in the same manner, the favored chair forms of the L-series of sugars generally have a carbon atom sequence which follows a counterclockwise order, and the ring conformation is therefore $1C_{(L)}$. This system for deriving a pyranose ring conformation (Shallenberger and Acree 1971) is similar to that proposed by Schwarz (1967) and Szejtli (1969), and can also be used for deducing the structure of furanose conformations. The system has the advantage of bringing the molecular rotations of the sugars into accord with the actual shape of their rings. For example, β-D-arabinose, α-L-galactose, and β-D-fructose are all assigned 1C as the favored conformation, which is in accord with their molecular rotations of -28,610, -27,150 and -23,820, respectively.

Another advantage of such a system is that the ideas of configuration and conformation are distinct and separate, and a change in configuration cannot alter the conformational description. The β-anomeric form of a sugar can be defined as that compound in which the anomeric OH group is equatorially disposed in the 1C conformation of the L-series of sugars and in the C1 conformation of the D-series. In these series and conformations, the anomeric OH group of the α-anomer is axially disposed.

Other conformations possible for pyranose rings are *half-chair* and *sofa* forms (Durette and Horton 1971). In the former, 4 of the atoms of the pyranoid ring are constrained to coplanarity, and in the latter, 5 of the 6 atoms are coplanar:

HALF-CHAIR CONFORMATIONS

Furanose Conformational Isomers.—For many years the furanose rings of the sugars were believed to be planar, but it is now recognized that there are several puckering modes for the furanose ring (Hall 1963). The puckering modes lead to two basic conformations for the furanoid ring called the *twist* and the *envelope* conformations. Their shapes, in perspective, are:

ENVELOPE CONFORMATION TWIST CONFORMATION

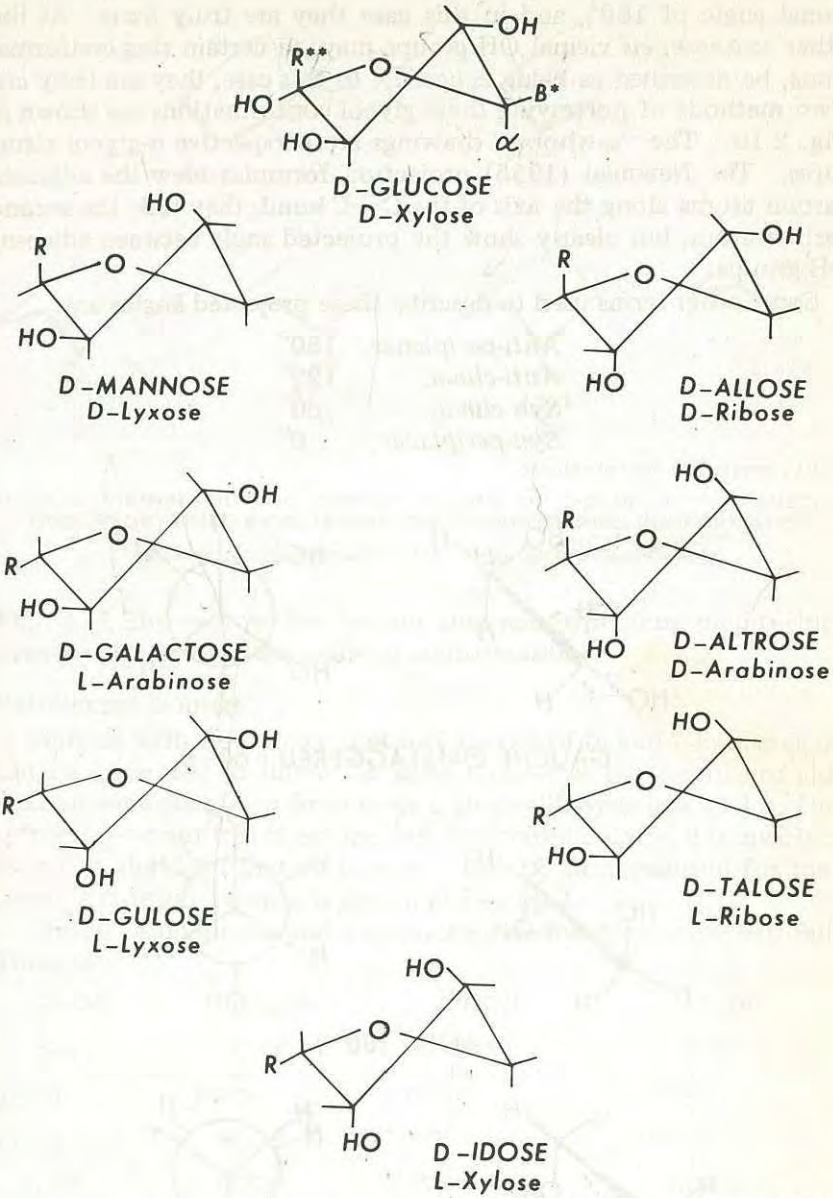
To specify a twist conformation, three atoms are used to describe the plane for the ring. The letter "T" is used with a superscript and a subscript to show the carbon atoms displaced "above" and "below" the plane of the ring. The twist conformation shown is the T_2^3 conformation. In an envelope conformation, 4 atoms describe a plane, and the conformation shown above is V^1. For convenient reference, the T_3^2 conformations for aldohexofuranose and aldopentofuranose sugars are shown in Fig. 2.9.

All told, there are ten forms possible for the envelope and the twist conformations, respectively. Chiral designations are required since, for example, if the T_3^2 conformation is favored for a D-sugar, the favored conformation for the L-furanoid sugar is T_2^3. While those particular twist conformations shown in Fig. 2.9 are not necessarily favored, that shown for β-D-galactofuranose would seem to be particularly favorable, since all carbon atom substituents are equatorially disposed.

Vicinal Glycol Conformations.—When the shape of the pyranose and furanose ring is altered, the relative disposition of each OH substituent, with respect to the shape of the ring, and to each other, is also altered. To describe the conformation of vicinal OH (α-glycol) groups, the following terms are used.

When the torsion (projected) angle between vicinal OH groups is 60°, the glycol conformation is described as *gauche* or *staggered*. In the favored chair conformation for β-D-glucopyranose, the disposition of all OH groups is staggered, and the torsional angle for adjacent OH groups is 60° in all cases. They are equidistant regardless of whether they are configurationally *cis* or *trans*. This aspect of sugar structure is the basis for statements to the effect that there are seldom true *cis* and *trans* relations in the sugar series.

When vicinal OH groups are *trans* in certain conformations, the OH groups may assume the *anti* glycol conformation, which has a tor-



Shallenberger and Acree (1971)

FIG. 2.9. T_3^2 CONFORMATIONS FOR D-SERIES OF ALDOHEXOFURANOSES AND ALDOPENTOFURANOSES, AND ALSO FOR L-SERIES OF ALDOPENTOFURANOSES

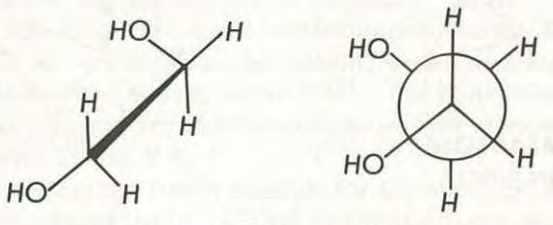
*—Anomeric designation is reversed for L-series of pentoses.
**—R = —CH_2OH for pentoses and —CHOH—CH_2OH for hexoses (capitalized names).

Reproduced with permission of Springer-Verlag.

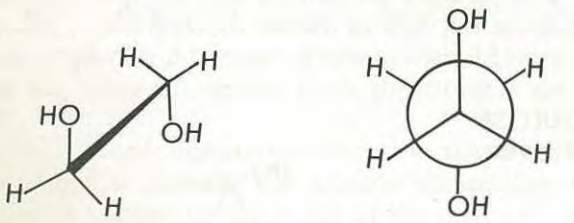
sional angle of 180°, and in this case they are truly *trans*. At the other extreme, *cis* vicinal OH groups may, in certain ring conformations, be described as being *eclipsed*. In this case, they are truly *cis*. Two methods of portraying these glycol conformations are shown in Fig. 2.10. The "sawhorse" drawings are perspective α-glycol structures. The Newman (1955) projection formulas view the adjacent carbon atoms along the axis of the C—C bond; they hide the second carbon atom, but clearly show the projected angle between adjacent OH groups.

Some other terms used to describe these projected angles are:

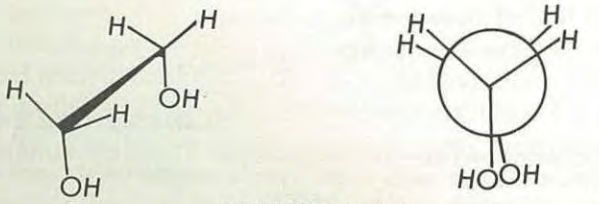
Anti-periplanar,	180°
Anti-clinal,	120°
Syn-clinal,	60°
Syn-periplanar,	0°



GAUCHE OR STAGGERED (60°)

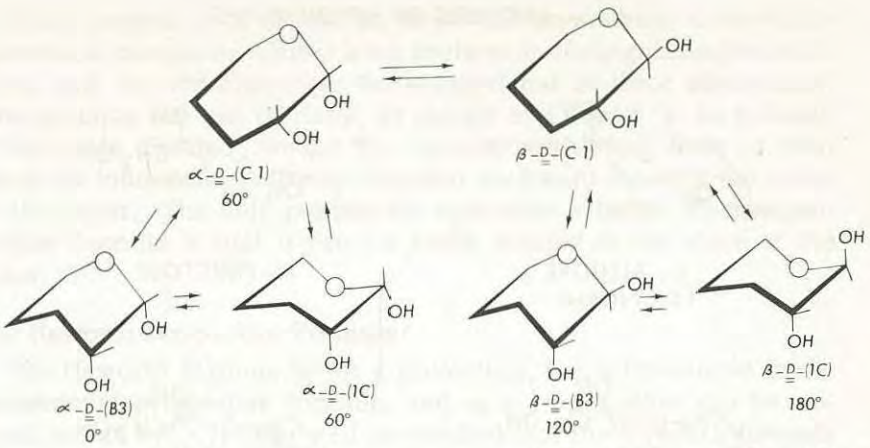


ANTI (180°)



ECLIPSED (0°)

FIG. 2.10. SAWHORSE AND NEWMAN PROJECTION FORMULAS FOR VARIOUS VICINAL GLYCOL CONFORMATIONS



Shallenberger and Acree (1969)

FIG. 2.11. VICINAL GLYCOL CONFORMATIONS OF D-GLUCOSE OH_1 AND OH_2 GROUPS OBTAINED BY ALTERING THE PYRANOSE RING CONFORMATION

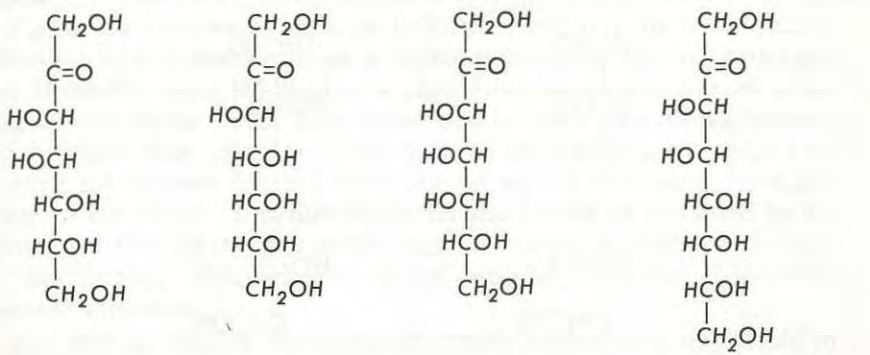
Reproduced with permission of the American Chemical Society.

Fig. 2.11 shows how the various ring conformations of β-D-glucopyranose lead to various α-glycol conformations.

Ketohexose Isomers

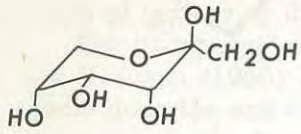
Starting with dihydroxy acetone, a series of D- and L-ketose sugars can be generated in much the same manner as the D-series of aldohexoses were generated from D- or L-glyceraldehyde in Fig. 2.3. Only D-fructose occurs free in nature, and in glycosidic union it is invariably found as the β-D-furanose isomer. The 1C conformation for the 4 possible D-ketopyranoses is shown in Fig. 2.12.

Several ketoheptoses and a ketooctose are found to occur naturally. These are:

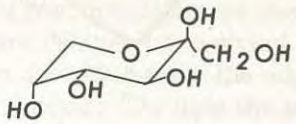


D-*MANNO*-HEPTULOSE | D-*ALTRO* HEPTULOSE | D-*TALO* HEPTULOSE | D-*GLYCERO*-D-*MANNO*-OCTULOSE

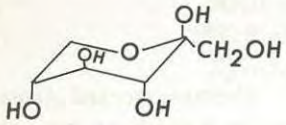
β-D- ANOMERS OF KETOHEXOSES



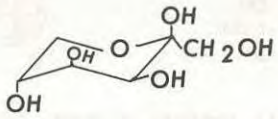
D- ALLUOSE
(D- Psicose)



D- FRUCTOSE



D- SORBOSE



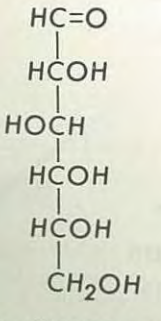
D- TAGATOSE

FIG. 2.12. THE 1C CONFORMATION FOR THE β-D-ANOMERS OF THE 4 POSSIBLE KETOHEXOSES

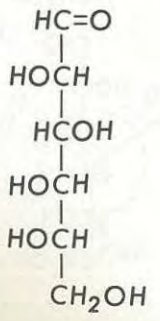
PERMISSIBLE OPERATIONS WITH STRUCTURAL FORMULAS FOR THE SUGARS

The Fischer Projection Formula

The Fischer projection formula is a *projection* of a three-dimensional structure into two dimensions, and as a consequence the spatial direction of carbon atom substituents is not indicated but "understood" to be projected from the plane of the paper toward the viewer. Therefore, the structure may never be *lifted* out of the plane of the paper and turned over because the resulting structure would then be identical with the structure used to depict the enantiomer.



D-GLUCOSE



L-GLUCOSE

If the projection is altered to show the asymmetric carbon substituents in perspective, there is no problem in distinguishing enantiomers, and the structures can be manipulated in three dimensions. This requires the use of darts, as shown in Chapter 1, to indicate substituents directed toward the viewer, and dotted lines or hash marks to indicate substituents directed backward through the plane of the paper. The only permissible operation with the Fischer projection formula is that it can be freely rotated in the plane of the paper.

The Haworth Perspective Formula

The Haworth formula is not a projection, but a formalized three-dimensional perspective formula, and as a consequence can be manipulated at will. It is so well established that the Haworth formula is a perspective formula that it is permissible to show the structure without shading the lower bonds of the ring to indicate that they project toward the reader; caution must be exercised, however. A recently published mechanism to show how D-glucose is transported across a biological membrane employs the biologically inert L-enantiomer in a stereochemical diagram, and a recent elucidation of the stereochemistry of β-D-glucose in the casimidine structure erroneously shows the structure for β-L-glucose.

As shown in Figs. 2.13 and 2.14 there is adequate distinction between enantiomers regardless of their manipulation, such as turning the structure over or rotating it in the plane of the paper. In Fig. 2.13 α-D-glucopyranose I is lifted from the plane of the paper and turned over, from either left to right or right to left (1) to yield II, which yields IV when rotated 45° (2) in the plane of the paper. Structure III results when I is lifted from the plane of the paper and turned over from back to front (3) and IV, upon rotating 90° in the plane of the paper.

Figure 2.14 shows α-L-glucose in form I, which is the mirror-image structure of α-D-glucose when a mirror is placed above the structure, and II results when the mirror is placed below, or α-D-glucose is sitting upon a mirror. Structure II can also be generated by transposing the configuration of α-D-glucose I at all asymmetric centers, or by turning α-L-glucose I over, from left to right by lifting it from the plane of the paper (1). Structures III and IV can be obtained by rotation and turning the structures over. Whenever the latter operation is performed, the carbon atom number sequence becomes counterclockwise.

The key to writing Haworth structural formulas in any position (for example, to accommodate a nucleoside attachment) without

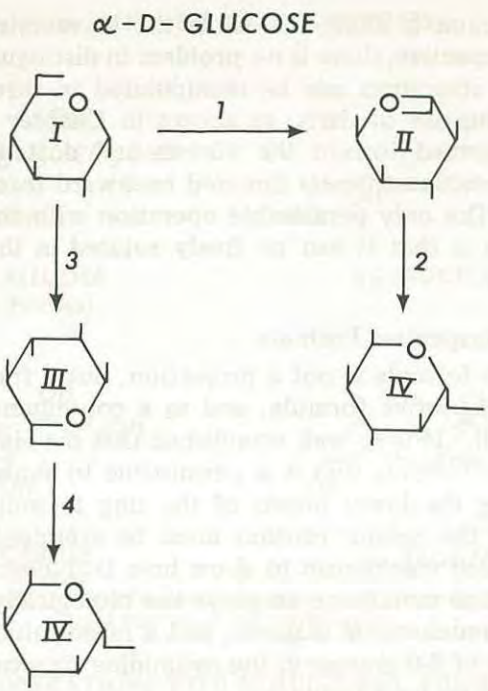


FIG. 2.13. PERMISSIBLE OPERATIONS WITH THE HAWORTH PERSPECTIVE FORMULA FOR α-D-GLUCOPYRANOSE

altering their identity is to remember that turning a structure over places substituents that were on the "top" of the molecule on the "bottom," and vice versa. The sugar may then be rotated in any direction and to any degree. Identification of a structure or of an unknown sugar requires manipulation so that a form similar to I is generated. No matter what operation one performs on the Haworth perspective structures, there is no structure for one enantiomer that is superposable on the other.

Unfortunately, authoritative directions for manipulating sugar structures (Anon. 1948) specifically state that the Haworth formula cannot be rotated "because it is not a projection." The statement and the reason are in error. The Haworth structure can be rotated and even turned over, *because* it is not a projection, but rather a perspective structure.

The Conformational Formula

The manipulations permissible with the Haworth perspective formula, discussed earlier in this chapter, are also permitted with the

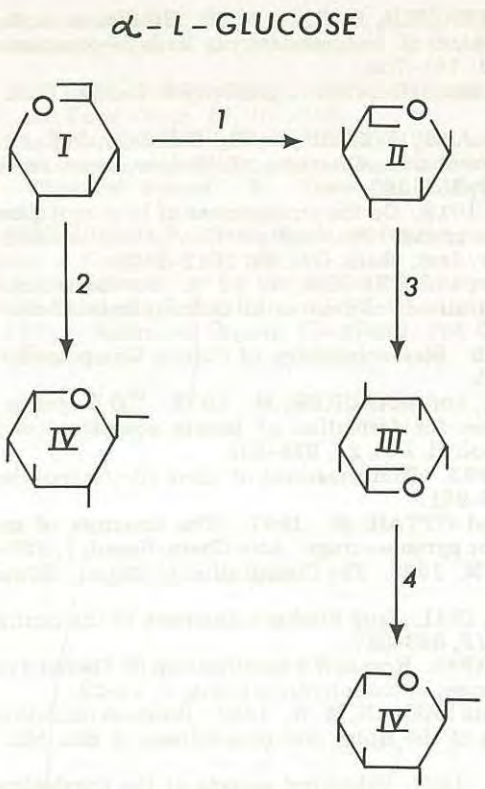


FIG. 2.14. PERMISSIBLE OPERATIONS WITH THE HAWORTH PERSPECTIVE FORMULA FOR α-L-GLUCOPYRANOSE

conformational formulas of the sugars; here, however, the ring is puckered and the ring bonds projecting "upward" from the average plane for the ring must be drawn downward when the ring is turned over, and vice versa. The same holds true for carbon atom substituents, but it is easier to remember that axial and equatorial dispositions are *not* altered.

BIBLIOGRAPHY

ACREE, T. E. 1965. Conformational interpretation of the sugar-borate reaction. M.S. thesis (unpublished). Cornell University.

ACREE, T. E. 1973. The chemistry of sugars in boric acid solutions. *In* Carbohydrates in Solution, R. F. Gould, Editor. Advances in Chemistry Series *117*, 208-219.

ANGYAL, S. J. 1969. The composition and conformation of sugars in solution. Angew. Chem. (International Edition) *8*, 157-166.

ANON. 1948. Rules of carbohydrate nomenclature. Chem. Eng. News, 1623-1628.

BAER, E., and FISCHER, H. O. L. 1939. Studies on acetone-glyceraldehyde. VII. Preparation of l-glyceraldehyde and l-(-)-acetone glycerol. J. Am. Chem. Soc. *61*, 761–765.

BENTLEY, R. 1969. Molecular Asymmetry in Biology, Vol. I. Academic Press, New York.

BRIMACOMBE, J. S., FOSTER, A. B., STACEY, M., and WHIFFEN, D. H. 1958. Aspects of stereochemistry. I. Properties and reactions of some diols. Tetrahedron *4*, 351–360.

BÖESEKEN, J. 1913. On the arrangement of hydroxyl groups of polyhydroxy compounds in space. The configuration of the saturated glycols of α- and β-glucose. Ber. deut. chem. Ges. *46*, 2612–2628.

DURETTE, P. L., and HORTON, D. 1971. Conformational analysis of sugars and their derivatives. Advances in Carbohydrate Chem. and Biochem. *26*, 49–125.

ELIEL, E. 1962. Stereochemistry of Carbon Compounds, McGraw-Hill Book Co., New York.

GORIN, P. A. J., and MAZUREK, M. 1973. ^{13}C Magnetic resonance spectroscopic evidence for formation of borate complexes of polyhydroxy compounds. Carbohyd. Res. *27*, 325–339.

HALL, L. D. 1963. Conformations of some ribofuranosides. Chem. and Ind. (London) 950–951.

HASSEL, O., and OTTAR, B. 1947. The structure of molecules containing cyclohexane or pyranose rings. Acta Chem. Scand. *1*, 929–942.

HAWORTH, W. N. 1928. The Constitution of Sugars. Edward Arnold and Co., London.

HUDSON, C. S. 1941. Emil Fischer's discovery of the configuration of glucose. J. Chem. Ed. *18*, 353–357.

HUDSON, C. S. 1948. Rosanoff's modification of Fischer's proposals of nomenclature. Advances in Carbohydrate Chem. *3*, 12–22.

ISBELL, H. S., and PIGMAN, W. W. 1937. Bromine oxidation and mutarotation measurements of the alpha- and beta-aldoses. J. Res. Nat. Bur. Std. *18*, 141–194.

LARDER, D. F. 1967. Historical aspects of the tetrahedron in chemistry. J. Chem. Ed. *44*, 661–666.

LOWREY, T. M. 1935. Optical Rotatory Power. Longmans, Green and Co., London.

MACPHERSON, H. T., and PERCIVAL, E. G. V. 1937. Investigations on the configuration of carbohydrates by conductivity measurements in boric acid solution. J. Chem. Soc. 1920–1927.

MAZUREK, M., and PERLIN, A. S. 1963. Borate complexing by five-membered ring *vic*-diols. Vapor pressure equilibrium and N.M.R. spectral observations. Can. J. Chem. *41*, 2403–2411.

NEWMAN, M. S. 1955. A notation for the study of certain stereochemical problems. J. Chem. Ed. *32*, 344–347.

PASTEUR, L. 1860. Studies on the dissymmetry of molecules in natural organic products. Lecons Chimique de Paris. Society Chimique de Paris. Alembic Club Reprint 14.

REEVES, R. 1949. Cuprammonium-glycoside complexes. III. The conformation of the D-glucopyranoside ring in solution. J. Am. Chem. Soc. *71*, 215–217.

ROSANOFF, M. A. 1906. On Fischer's classification of stereoisomers. J. Am. Chem. Soc. *28*, 114–128.

SACHSE, H. 1890. On the geometry of isomers of hexamethylene derivatives. Ber. deut. chem. Ges. *23*, 1363–1370.

SCHWARZ, J. P. C. 1967. Cited by L. Hough and A. C. Richardson. *In* Rodd's Chemistry of Carbon Compounds, S. Coffey (Editor, Vol. I, part F). Elsevier, Amsterdam.

SHALLENBERGER, R. S., ACREE, T. E., and GUILD, W. E. 1965. Configuration conformation and sweetness of hexose anomers. J. Food Sci. *30*, 560-563.

SHALLENBERGER, R. S., and ACREE, T. E. 1969. Molecular structure and sweet taste. J. Agr. and Food Chem. *17*, 701-703.

SHALLENBERGER, R. S., and ACREE, T. E. 1971. Chemical structure of compounds and their sweet and bitter taste. *In* Handbook of Sensory Physiology. IV. Chemical Senses. 2. Taste, L. M. Beidler (Editor). Springer-Verlag, Berlin.

SICKLES, J. P., and SCHULTZ, H. P. 1964. Evidence for the configuration on carbon 1 of D-glucose. J. Chem. Ed. *41*, 343-345.

SZEJTLI, J. 1969. A new formula for the description of the conformations of pyranoid sugars. Acta Chim. Acad. Sci. Hung. *61*, 57-68.

WHELAND, G. W. 1960. Advanced Organic Chemistry, 3rd Edition. John Wiley & Sons, New York.

Occurrence and Properties of Sugars

Sugars and sugar polymers are synthesized in plants and used by plants and animals as convenient sources of energy. A metabolic relationship exists between the amount of sugar in an organism and its stored condensed energy form, polysaccharide. The organism produces more or less sugar out of the polysaccharide energy store according to its environment and stage of growth. Thus many of the published figures for carbohydrate distribution in organisms are subject to wide variation, and can only be considered a rough guide to the amounts to be found in different organisms.

D-Glucose is by far the most widely distributed sugar in nature and in fact it is the D-forms of the sugars that are usually encountered, although L-rhamnose, L-arabinose, and L-galactose are also known. Trioses such as glyceraldehyde and dihydroxy acetone, are important metabolic intermediates in the Embden-Meyerhof-Parnas pathway of glycolysis (as phosphorylated compounds), and D-erythrose phosphate, a tetrose, is important in some organisms. Heptoses and their derivatives also occur, but the most widespread sugars are the hexoses and pentoses.

TYPES OF SUGAR

General Distribution

Naturally occurring sugars may contain different numbers of carbon atoms, different functional groups or substituents, and may be simple or composite. Thus aldoses, ketoses, deoxy sugars, amino sugars, acetylated or benzoylated sugars, methylated sugars, sugar acids, glycosides, oligosaccharides, and anhydro sugars are encountered.

Most sugars are classified as aldoses or ketoses since the aldehydo or keto group must be present in all of them—either free, in hemiacetal form, or in the full acetal, glycosidically combined form. The most significant occurrence of a deoxy sugar is 2-deoxy ribose, which is a component of deoxyribonucleic acid. Amino sugars occur as components of slimes, mucosubstances, antibiotics, and in the insect and crustacean polymer, chitin. Various glycosides are ubiquitously distributed in the plant and animal kingdoms. The nonsugar portion of the glycosides (the aglycone) is usually an important metabolite; one of the functions of the glycone moiety is believed to be that it

lends an element of water solubility to an otherwise insoluble metabolite. It is also probable that the receptor site for the metabolite is made up of both hydrophobic and hydrophilic components.

Many glycosides are valuable therapeutically as drugs, for example, the cardiac glycosides. Most naturally occurring glycosides that have a nonsugar moiety are the β-D-glucopyranosides. Examples are salicin (*O*-hydroxymethyl phenyl-β-D-glucoside), an analgesic obtained from the willow (Salix), and anthocyanin pigments, which are coloring substances.

The reason for the occurrence of acetyl and benzoyl esterification or methyl etherification products of the sugars is obscure, and in any case, reports of such derivatives are few in number. Sugar alcohols such as sorbitol are by no means widespread, but may occur in significant amounts in fruits. Anhydro sugars are rare in the free state, but are widely distributed in combined form in many seaweed polysaccharides such as agar and alginates. They are also present in certain processed foods. The oligosaccharides present an extremely large class of naturally occurring sugars, and will subsequently be dealt with in detail.

Pentoses and Hexoses

Of the 8 possible D- and L-aldopentoses, only 4 occur naturally, namely, D-xylose, D-ribose, and D- and L-arabinose, and are mostly present as constituents of polysaccharides. For example, xylose, the most prevalent pentose is a constituent of the xylan called holocellulose, which is present in wood gum, cottenseed hulls and in corn cobs. It occurs in β-D-glucosidic linkage and in the C1 conformation, has the same "all equatorial" distribution of hydroxyl substituents that is found in the most prevalent hexose, β-D-glucose, also in the C1 conformation.

The aldopentose lyxose is not found naturally. D-Ribose occurs in the furanose form in glycosidic linkage in compounds such as ribonucleic acid. It is not found in the free state. Arabinose occurs in the free state in small amounts, and both D- and L-forms are known; however, it is mostly encountered as a glycosidic constituent of hemicellulose, a structural material of plants, gums, and pectins.

Of the 8 aldo-D-hexoses possible (16, if anomers are counted), only one occurs abundantly in nature, i.e., D-glucose. Trace amounts of D-galactose and D-mannose occur, but these sugars are chiefly found in combined form. D-galactose occurs in lactose and mannose in the polysaccharide mannan, a gummy plant exudate. Ketoses also occur in the free state, but only one, D-fructose, is found to any great extent. It is the major constituent also of the polysaccharide

inulin, and is one of the glycosidic hexose moieties of the abundant disaccharide, sucrose.

Oligosaccharides

When from 2 to 9 or 10 monosaccharide units are combined glycosidically, the carbohydrate is called an oligosaccharide. If 10 or more monosaccharides are joined, the compound is called a polysaccharide. Although about 40 oligosaccharides are found free in nature, only a few are present in abundance in foods, namely:

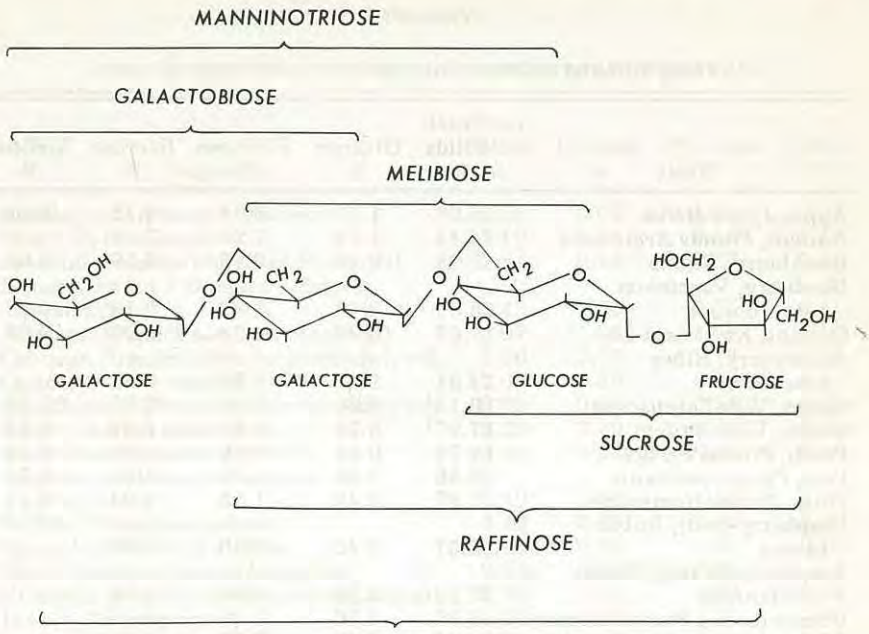
Sucrose (α-D-Glucopyranosyl β-D-fructofuranoside)
Lactose (4-*O*-β-D-Galactopyranosyl-D-glucopyranose)
Maltose (4-*O*-α-D-Glucopyranosyl-D-glucopyranose)
α,α-Trehalose (α-D-Glucopyranosyl-α-D-glucopyranoside)
Raffinose [*O*-α-D-Galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside]
Stachyose [*O*-α-D-Galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside]

Thus, the most abundant oligosaccharides are made up of only 3 monosaccharides, D-glucose, D-galactose and D-fructose.

Sucrose is the familiar sugar of commerce, obtained from sugar cane and the sugar beet. It is ubiquitously present in plants. Lactose is the sugar of mammalian milk, at a concentration of about 7.5% in human and 4.5% in cow's milk. It is, of course, also present in dairy products such as ice cream, yogurt, and buttermilk. The occasional claims that lactose is present in certain plants have not been verified. Closely related sugars, similar in properties, are probably mistaken for lactose. These sugars will be discussed shortly.

Maltose is not found in abundance in the free state except in sugar syrups produced by the action of amylase on starch. α,α-Trehalose is found in lower plants (fungi, yeasts, mushrooms).

The galactosylsucrose series of oligosaccharides deserves special attention. Raffinose, stachyose, and traces of verbascose may be the major sugar components of many foods, particularly those of legume origin. They are the only known plant oligosaccharides to occur in abundance which contain galactose, a fact that may have direct bearing on the nutritional quality of foods of plant origin. In addition, the galactosylsucroses contain component oligosaccharides which may also occur free in foods of plant origin. The constitution of stachyose, depicting its component mono-, di- and trisaccharides is:



In the free state, manninotriose, galactobiose, and melibiose are reducing sugars, and it is either melibiose or galactobiose that is probably mistaken for lactose in plants, since those plants in which lactose has been reported are known to contain stachyose. Verbascose is a pentasaccharide containing still another galactose monomer linked α-(1→6) to the terminal galactose unit of stachyose.

The occurrence of the α-(1→6) linkage is perhaps the unique feature of the galactosylsucroses. These uncommon glycosidic bonds lead to special utilization problems when they are ingested in quantity. Because they contain a sucrose unit which is easily hydrolyzed by acid or yeast invertase, the galactosylsucroses are often mistaken for or counted as sucrose in biological materials unless special precautions have been taken to establish the identity of individual sugars.

Distribution of Sugars in Plants

At the outset of this chapter, mention was made of the great variation in the sugar content which may be encountered in organisms, particularly in plants and foods of plant origin. Nevertheless, tables which give the approximate content and distribution of sugars are useful. Tables 3.1, 3.2 and 3.3 are a summary of data compiled

TABLE 3.1

FREE SUGARS IN FRUIT AS PERCENTAGE FRESH BASIS

Fruit	Total Solids %	Glucose %	Fructose %	Sucrose %	Maltose %
Apple, *Pyrus Malus*	15.96	1.17	6.04	3.78	Trace
Apricot, *Prunus Armeniaca*	14.44	1.73	1.28	5.84	
Blackberry, *Rubus*	15.28	2.48	2.15	0.59	0.66
Blueberry, *Vaccinium corymbosum*	15.89	3.76	3.82	0.19	0.08
Currant, *Ribes sativum*	17.68	3.33	3.68	0.95	0.64
Gooseberry, *Ribes grossularia*	14.81	3.29	3.90	1.21	
Grape, *Vitis Labruscana*	19.13[1]	6.86	7.84	2.25	1.58
Grape, *Vitis vinifera*	17.97[1]	5.35	5.33	1.32	2.19
Peach, *Prunus Persica*	12.79	0.91	1.18	6.92	0.12
Pear, *Pyrus communis*	13.58	0.95	6.77	1.61	0.31
Plum, *Prunus domestica*	17.97	3.49	1.53	4.94	0.15
Raspberry (red), *Rubus idaeus*	20.67	2.40	1.58	3.68	
Raspberry (black), *Rubus occidentalis*	28.22	4.56	4.84	1.90	
Cherry (sour), *Prunus cerasus*	15.05	4.30	3.28	0.40	
Cherry (sweet), *Prunus avium*	22.39	6.49	7.38	0.22	
Strawberry, *Fragaria chiloensis*	9.45	2.09	2.40	1.03	0.07

[1] Soluble solids.

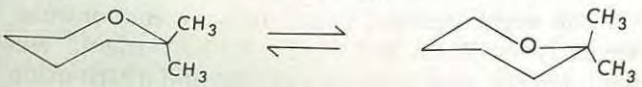
by Lee *et al.* (1970), with omission of the occasional occurrence of trace amounts of rare sugars.

GENERAL PHYSICAL PROPERTIES

Optical Rotation

When the structure of a compound is not superposable upon its mirror-image structure, it usually possesses optical activity. The elements of chirality in a molecule may be atomic (configurational) or conformational. As discussed in Chapter 2, when the tetrahedral carbon atom has four different substituents, *a*, *b*, *c*, and *d*, it is made asymmetrical and it cannot be superposed upon its mirror image. This is an example of configurational asymmetry.

To demonstrate conformational asymmetry, the mirror-image ring structures of 2,2-dimethyltetrahydropyran are used:



2,2-DIMETHYLTETRAHYDROPYRAN

TABLE 3.2

FREE SUGARS IN VEGETABLES AS PERCENTAGE FRESH BASIS

Vegetable	Total Solids %	Glucose %	Fructose %	Sucrose %
Asparagus, *Asparagus officinalis*	9.15	0.92	1.30	0.28
Beet,[1] *Beta vulgarus*	11.19	0.18	0.16	6.11
Broccoli, *Brassica oleraceae (botrytis)*	11.84	0.73	0.67	0.42
Brussels sprout,[1] *Brassica oleracea (gemmifera)*	11.45	0.66	0.75	0.41
Cabbage, *Brassica oleracea (capitata)*	6.67	1.58	1.20	0.15
Cabbage, *Brassica oleracea (capitata)*, red	9.06	2.06	1.74	0.50
Carrot, *Daucus carota*	12.00	0.85	0.85	4.24
Cauliflower,[1] *Brassica oleracea (botrytis)*	8.05	0.83	0.74	0.67
Celery, *Apium graveolens*	8.29	0.49	0.43	0.31
Cucumber, *Cucumis sativus*	3.46	0.86	0.86	0.06
Eggplant, *Solanum melongena (esculentum)*	8.49	1.51	1.53	0.25
Endive, *Cichorum endivia*	5.60	0.07	0.16	0.07
Escarole, *Cichorum endivia*	6.15	0.16	0.32	0.10
Kale, *Brassica oleracea (acephala)*	9.74	0.27	0.21	
Kohlrabi, *Brassica oleracea (gongylodes)*	7.55	1.34	1.24	0.58
Leek,[1] *Allium porrum*	11.95	0.98	1.47	1.06
Lettuce, *Lactuca sativa*	4.97	0.25	0.46	0.10
Melon, Honeydew, *Cucumis melo*	12.74	2.56	2.62	5.86
Melon, Musk, *Cucumis melo (reticulatus)*	10.84	1.72	2.03	3.56
Melon, Water, *Citrullus vulgarus*	9.57	1.81	3.54	2.35
Okra, *Hibiscus esculentus*	10.70	1.03	1.06	0.75
Onion,[2] *Allium cepa*	11.56	2.07	1.09	0.89
Onion, green,[1] *Allium cepa*	9.59	0.56	0.76	0.86
Parsley, *Petroselinum hortense*	11.28	0.10		0.20
Parsnip,[1] *Pastinaca sativa*	20.99	0.18	0.24	2.98
Pepper, *Capsicum frutescens*	6.21	0.90	0.87	0.11
Potato, new, *Solanum tuberosum*	20.08	0.15	0.09	0.14
Potato, stored at 35°F[1]		1.04	1.15	1.69
Pumpkin,[1] *Cucurbita pepo*	7.13	1.69	1.43	1.30
Radish, white, *Raphanus sativus*	4.40	0.84	0.30	
Radish, red, *Raphanus sativus*	5.46	1.34	0.74	0.22
Rhubarb, *Rheum rhaponticum*	6.20	0.42	0.39	0.09
Rutabaga, *Brassica napobrassica*	6.69	0.38	0.34	0.07
Spinach, *Spinacia oleracea*	8.04	0.09	0.04	0.06
Squash, summer, *Cucurbita pepo*	5.55	0.77	0.82	0.09
Squash, winter, *Curcurbita pepo*	13.08	0.96	1.16	1.61
Sweet corn, *Zea mays*	22.69	0.34	0.31	3.03
Swiss Chard, *Beta vulgaris (cicla)*	9.20	0.17	0.09	0.06
Sweet Potato, *Ipomoea batatas Poir*	22.53	0.33	0.30	3.37
Tomato, *Lycopersicon esculentum*	5.23	1.12	1.34	0.01
Turnip, *Brassica rapa*	7.40	1.50	1.18	0.42

[1] Contains traces (0.02–0.20%) raffinose, stachyose, or both.
[2] Contains 0.24% to > 1.0% raffinose and stachyose.

TABLE 3.3

FREE SUGARS IN LEGUMES

Legume	Total Solids	Glucose	Fructose	Sucrose	Raffinose	Stachyose
Fava bean,[1] *Vicia faba*	16.61		0.18	3.36	0.66	
Lima bean,[1] *Phaseolus lunatus*	26.74	0.04	0.08	2.59	0.20	0.59
Pole Lima bean[1], *Phaseolus lunatus*	24.58	0.18		2.26	0.32	0.60
Pole snap bean,[1] *Phaseolus lunatus*	10.21	0.48	1.30	0.28	0.26	
Snap bean,[1] *Phaseolus vulgaris*	7.79	1.08	1.20	0.25	0.11	0.19
Pea (Alaska)[1] *Pisum sativum*	25.54		0.08	3.00	0.06	0.06.
(Wrinkled)[1] *Pisum sativum*	22.77	0.32	0.23	5.27	0.58	0.49
Cow Pea,[1] *Vigna sinensis*	39.30	0.08	0.06	1.86	0.10	1.66
Dry bean,[2] *Phaseolus vulgaris*				2.40	0.80	3.40
Mung bean,[2] *Phaseolus aureus*				1.19	0.40	1.75
Pea bean,[2] *Phaseolus vulgaris*				2.55	0.65	3.06
Pea seed,[2] *Pisum sativum*		0.24		4.11	1.75	7.96
Soybean,[2] *Glycine Max*				4.53	0.73	2.73

[1] Sugars as % fresh basis.
[2] Sugars as % total bean weight.

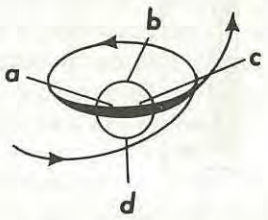
In spite of the fact that this compound does not possess an asymmetric carbon atom, it cannot be superposed upon its mirror-image structure because it does not possess an Sp axis of symmetry. According to Jaffee and Orchin (1965), when a molecule is rotated on an axis, and the resulting orientation is reflected in a plane perpendicular to that axis, the molecule has an Sp axis of symmetry if the resulting orientation is superposable on the original.

By present usage in organic chemistry, the two chair structures shown for 2,2-dimethyltetrahydropyran are considered to be *one* compound which, although they bear an enantiomeric relation to each other, are in rapid equilibrium and consequently optically inert. This example of conformational chirality was chosen, because of the analogy to the ring structures of the sugars. Along the same line of thought, the nearly eclipsed conformation of ethane would also be optically active;

ETHANE

however, ethane also exists as a "racemic" mixture of interchangeable conformations, and is, therefore, optically inert.

At this point in the discussion, it seems appropriate to bring up the fact that an atomic center that has four different substituents, where the highest atomic number precedes the lowest atomic number, or the highest order of polarizability precedes the lowest order of polarizability ($a > b > c > d$), is dextrorotatory or *rectus* (R) *because it has a left-handed screw pattern of polarizability*. The left-handed screw pattern of polarizability for a *dextro* configuration is shown below, and was proved by Brewster (1959) by mathematical calculation:



The reason why a substance that has an overall left-handed configurational or conformational element of chirality is described as "dextrorotatory" is the result of convention. The subject is of great importance to the biological properties of the sugars and is therefore reviewed here.

Plane-polarized light consists of both left and right circularly polarized light whose electric fields rotate about the axis of wave propagation, describing right-handed and left-handed helical or screw structures. A right-handed circularly polarized wave of light is shown emanating from a light source in Fig. 3.1 and passing through a polarimeter tube containing a solution of a compound which has an overall right-handed element of chirality. Such chiral elements usually result in optical activity for the compound; i.e., it possesses unequal refractive indices for the right- and left-handed circularly polarized light. As shown in Fig. 3.1, the right circularly polarized wave travels through the solution faster than the left circular wave, which is not shown, and is bent to the right; however, the viewer observes a refraction to the left, and the compound is described as being *levorotatory*. Hence, dextrorotation always signifies a left-handed spiral of the beam of polarized light being refracted to the left by a compound or substance that possesses a left-handed element of chirality.

Until about 1930, two opposite conventions existed for specifying optical rotatory power. Chemists defined a dextrorotatory substance as producing a clockwise rotation of the plane of polarization as viewed from the eyepiece of the polarimeter. Physicists defined

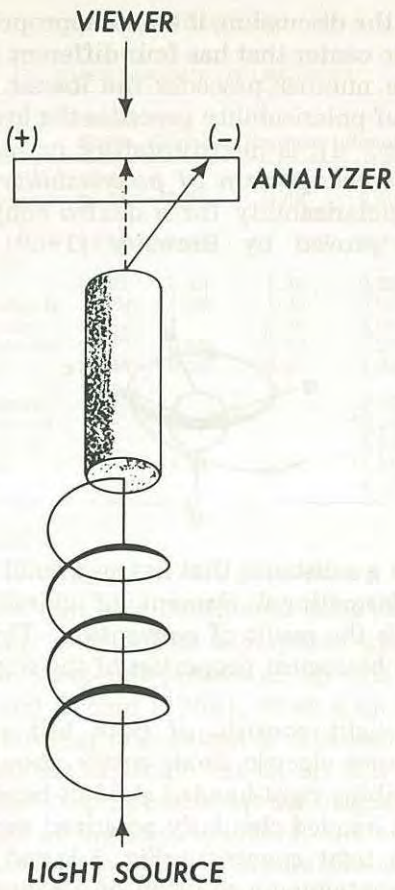


FIG. 3.1. EMANATION OF A RIGHT CIRCULAR SPIRAL OF PLANE-POLARIZED LIGHT PASSING THROUGH A SAMPLE TUBE CONTAINING AN OVERALL RIGHT-HANDED ELEMENT OF CHIRALITY, AND THE DIRECTION OF THE REFRACTION OF LIGHT (ROTATION) AS OBSERVED BY THE VIEWER

dextrorotation as that produced when viewed along the beam of light. The chemists' convention has now been adopted universally (Lowry 1935).

With the development of "conformational analysis" and the development of the idea of "favored conformations," Whiffen (1956) and Brewster (1959) established empirical ring conformational parameters for the sugars. Agreement between calculated and observed molecular rotations was particularly good when a sugar could be assumed to persist almost entirely in its favored chair conformation. Deviations

between the calculated and observed rotations were also used (Whiffen 1956) to estimate the concentration of the alternate chair conformation.

For a particular sugar, the individual contribution of atomic and conformational rotatory power cannot be easily separated in spite of the generalization (Eliel 1962) that conformational asymmetry usually takes precedence. Usually, however, as a consequence of the left-handed helical pattern of polarizability at the asymmetric carbon atom whereby ring closure takes place, the D-series sugars have an overall left-handed twofold screw pattern of polarizability for the ring structure alone. The D-series sugars are therefore usually dextrorotatory. Exceptions arise when the chiral family specification is assigned by reference to a center of asymmetry not involved in the formation of the ring, such as L-(+)-arabinose.

Specific and Molecular Rotation

A most useful tool for the study of sugar structures, or for the analysis of sugars, utilizes their optical rotatory power. When solutions of the sugars are observed in a polarimeter, they show a polarizing attribute that is characteristic for each sugar. At a given concentration, temperature, and wavelength, the specific rotation is the constant

$$[\alpha]_{\mathrm{D}}^{t} = \frac{\alpha}{c \times l}$$

where α is the observed rotation, t is the temperature, D the bright yellow line of sodium light, c the concentration of sugar in *grams per milliliter*, and l is the length of the polarimeter tube in decimeters.

The concentration of the sugar in solution is often expressed as p, or the parts, by weight, in a total of 100 parts of solution. The value p can be converted to c by multiplying p by the density of the solution. The specific rotation of important food sugars is shown in Table 3.4, calculated on an anhydrous basis. Usually, the specification of rotation for a crystalline form is given for the hydrate, and *not* on an anyhdrous basis. Where two values are given for a reducing sugar, one is that calculated from mutarotational data (Chapter 4) to represent the "initial" value. The second or final value is that found for mutarotational equilibrium.

Molecular rotation is the hypothetical rotation produced by the gram molecular weight of a compound dissolved in 1 ml of solution, and viewed in a 1-decimeter tube. For practical purposes, it is the specific rotation multiplied by the molecular weight of the compound. Since molecular rotations yield large values, they are usually,

but not always, divided by 100 and reported as such. The molecular rotation of a compound has theoretical significance for its structure. For example, molecular rotations were of special significance in deducing the anomeric configuration of the sugars.

Factors Affecting the Specific Rotation of Sugars.—*Concentration.*—The specific rotation of the sugars may vary greatly with concentration, and although Brown and Zerban (1941) have discussed this subject in detail, pertinent examples important to food sugar chemistry are given here.

The specific rotation of sucrose increases with increasing concentration from 5% to 15% solutions, and then decreases (Fig. 3.2). For dilute solutions (0.3% to 1%) the specific rotation decreases from about 68.5° to 66.9°; therefore, the specific rotation of sucrose must vary over a range of a full 2° between 1% and 4% sucrose concentration.

Glucose, as calculated from the equation

$$[\alpha]_D^{20} = 52.50 + 0.0227c + 0.00022c^2$$

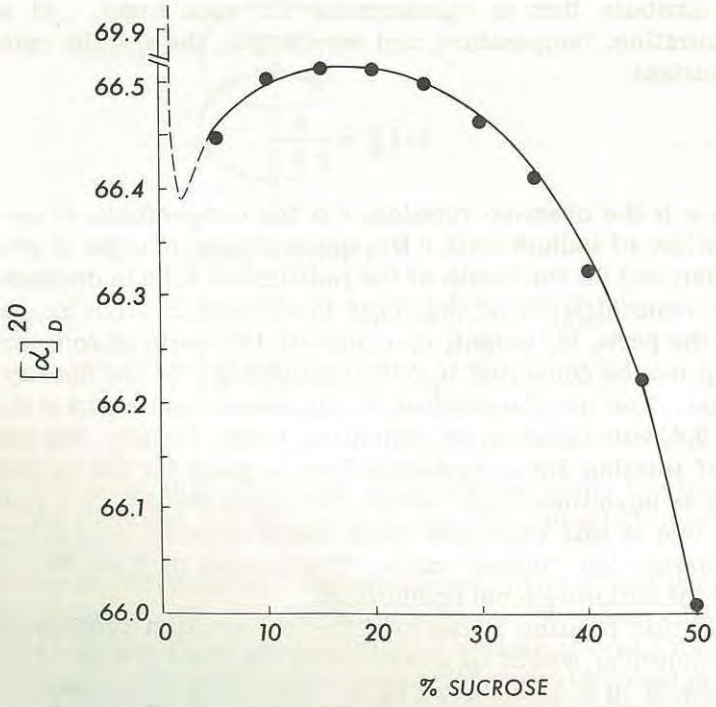


FIG. 3.2. EFFECT OF CONCENTRATION ON THE SPECIFIC ROTATION OF SUCROSE

has a specific rotation that increases from 52.51° at 1% concentration to 53.46° at 32% concentration, or by about 1°.

The specific rotation of fructose increases (becomes less levorotatory) with increase in concentration. The rate of increase is linear, and amounts to about 0.15° per 1% increase in sugar concentration.

Temperature.—Almost without exception, temperature increase favors the formation of the α-form of reducing sugars (Hudson 1909). Therefore, temperature change may significantly alter the specific rotation of sugars, particularly galactose, fructose, and invert sugar. After correcting for volume change, the specific rotation of sucrose has been found to decrease slightly with increasing temperature (0.01° per °C).

The specific rotation of fructose increases rapidly with increasing temperature, at a linear rate of about 0.7° per °C. Reports on the effect of temperature on the specific rotation of glucose are contradictory. The review by Brown and Zerban (1941) suggests that no change occurs over the range 1° to 100°C, but Hudson (1909) attributed a thermal lag in the specific rotation of glucose solutions to indicate that at elevated temperatures, α-D-glucose formation was favored. Isbell and Pigman (1937) report that the specific rotation of glucose at 0°C is 52.1°, while at 20°C it is 52.7°, and suggest that the difference is sufficiently large to require revision of the concept that glucose does not exhibit thermal mutarotation. Using a gas chromatographic procedure, an increase in specific rotation from 52.5° at 20°C to 56.8° at 90°C was found to be due to formation of the α-D-anomer at the expense of the β-D-anomer. The increase in the specific rotation of fructose was shown to be due to an increase in the concentration of the β-D-furanose isomer (Shallenberger 1973).

Sugar Mixtures.—The specific rotation of a given sugar is not appreciably affected by the presence of another sugar in the same solution. Lee *et al.* (1970) used this fact to validate the identity of individual sugars assayed in fruit and vegetable materials by comparing the observed specific rotation of extracts with that calculated from the contribution made by each individual sugar. In concentrated solutions the specific rotation of a mixture of sugars cannot be considered to be the sum of their specific rotations at the partial concentration of each sugar. Instead, the specific rotation of the mixture is the sum of the specific rotation that each sugar would have if it were present alone at a concentration equal to the total sugar concentration. In other words, the specific rotation of an equal mixture of glucose and fructose is equal to one-half the algebraic sum of the rotations of glucose and fructose at the same concentration.

Salts.—According to Murchauser (1923), alkali halides retard mutarotation and decrease the specific rotation of sugars. On the other hand, specific rotation is increased by alkaline-earth halides, but magnesium chloride is ineffective. Of particular interest to food chemists, Tomoda and Taguchi (1930) found that sodium bisulfite markedly lowers the polarizing power of glucose, arabinose, galactose and lactose, but has no effect on mannose, fructose, sucrose and raffinose. At about equal concentrations, bisulfite neutralizes the polarizing power of glucose.

Refraction and Spectral Properties

All carbohydrates are highly refractive, and although the refractive index varies according to the molecular type, this variation is not very great among the sugars. Consequently, the refractive index is related to the total amount of dissolved sugar, as shown in Fig 3.3.

Since sugars are colorless they do not absorb light of visible wavelength, and although they do absorb light in the ultraviolet region of the electromagnetic spectrum, the absorption band is too broad to

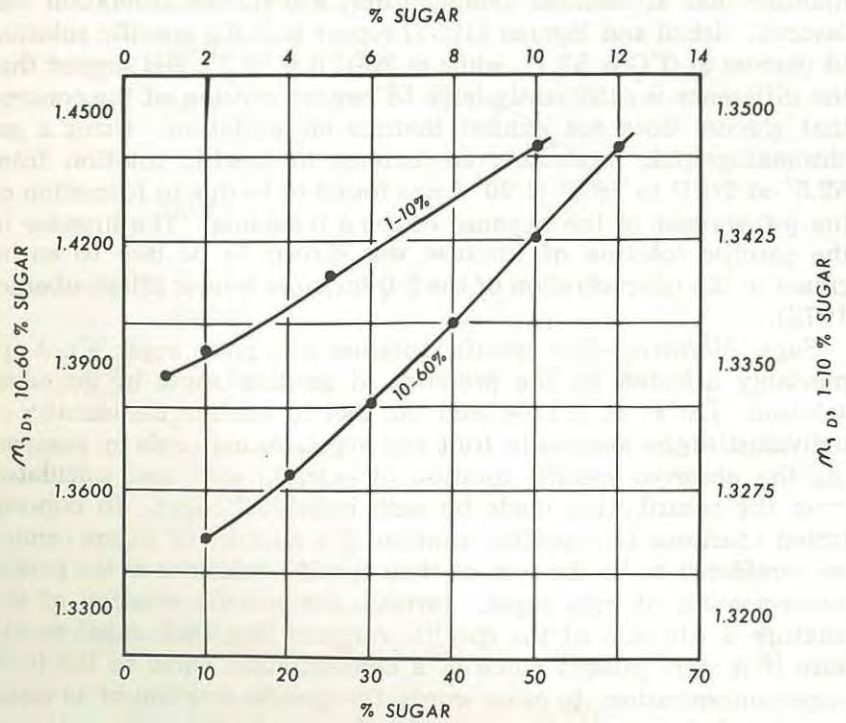
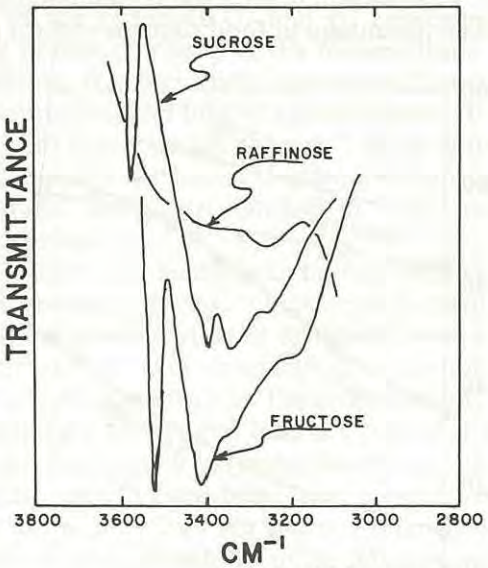


FIG. 3.3. REFRACTIVE INDEX OF SUGAR SOLUTIONS OF VARIOUS CONCENTRATIONS

permit assignment to a particular bond species. Infrared (IR) spectra can identify the tetrahydropyran ring, however, and the data obtained support the contention that the pyranoses persist in the stable chair conformation. The α-anomer of a pyranoid ring is reported to have a characteristic absorption band at about 844 cm^{-1}, and the β-anomer at about 891 cm^{-1} (Barker *et al.* 1954). Hydroxyl groups in different environments can also be identified by IR spectra. Sharp absorption bands in the region 3400 to 3600 cm^{-1} are due to free hydroxyl O—H stretching, while composite bands in the region 3200 to 3400 reciprocal centimeters are associated with hydrogen-bonded O—H stretching. When hydrogen bonding occurs, the OH absorption band shifts toward lower wave numbers and tends to become broader. An approximation of the hydrogen bond strength can be assessed by $\Delta\nu$, the difference between band positions for free and hydrogen-bonded OH groups.

The IR spectra of the hydroxyl region of some sugars is shown in Fig. 3.4. The sharp distinct bands for fructose and sucrose are assigned to free hydroxyl absorption, while broad and multiple bands are assigned to hydrogen-bonded OH groups (O—H . . . . O). The



Shallenberger (1963)

FIG. 3.4. INFRARED SPECTRA OF THE HYDROXYL REGION OF FRUCTOSE, SUCROSE AND RAFFINOSE USING THE NUJOL NULL TECHNIQUE

Reproduced with permission of the Institute of Food Technologists.

significant feature of these spectra is the very sharp free OH band for fructose, not matched by the spectra for any other sugar, and the absence of any free OH band for raffinose pentahydrate—a finding that seems to bear a relation to the low water solubility and lack of sweetness of this compound. The position of free OH bands, and the approximate position of hydrogen bonded OH bands for several sugars is given in Table 3.5. The data were obtained using the nujol null technique (Shallenberger 1963).

Solubility

All simple sugars and their simple derivatives are soluble in water, and most sugars have relatively high degrees of solubility. Values between 30% and 80% are quite common. The simple sugars are also fairly soluble in alcohol, but as molecular weight increases, solubility in alcohol falls off so markedly that precipitation from aqueous solution with alcohol constitutes a useful method for separating high molecular weight oligosaccharides from their lower molecular weight homologues, such as in the maltodextrin series (Birch *et al.* 1972).

The crystalline hydrates are usually less soluble than the anhydrous crystalline forms of the sugars, lactose monohydrate being a good example. The insolubility of the crystalline hydrates is, however, sometimes an advantage in food texture control (Birch 1972).

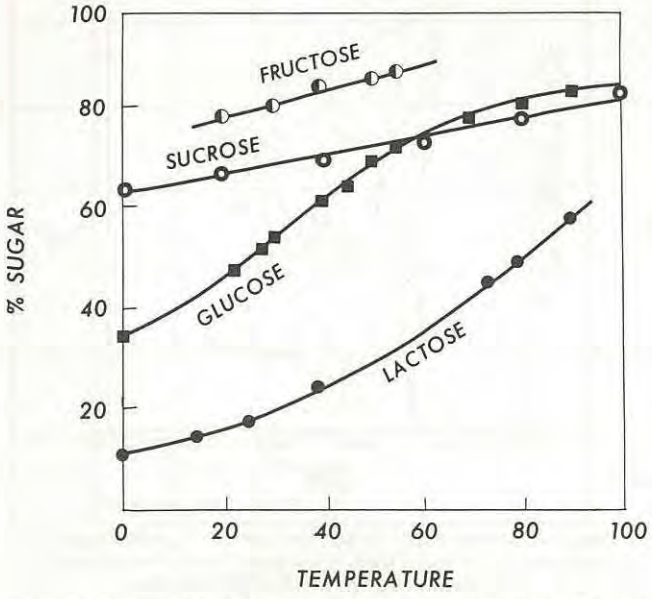


FIG. 3.5. APPROXIMATE SOLUBILITY OF VARIOUS SUGARS AT DIFFERENT TEMPERATURES

In contrast to water and the lower molecular weight alcohols, sugars have very low solubilities in nonpolar solvents. In fact, among the commonly available solvents, only pyridine, formamide, dimethylformamide, and dimethylsulfoxide show any tendency to dissolve them.

The approximate solubility of important food sugars at different temperatures is shown in Fig. 3.5. For reducing sugars, equilibrium between crystals and solution cannot be attained until mutarotation is complete. The solubility of a sugar in a solution containing another sugar is diminished.

Crystallization

Most sugars can be induced to crystallize, particularly the nonreducing oligosaccharides. However, it is difficult to obtain certain reducing sugars in crystalline form because the presence of anomers and ring isomers in solution make the reducing sugars intrinsically "impure." The presence of ring isomers hinders the crystallization of fructose, and an unstable conformation, leading to significant amounts of conformational isomers in equilibrium solution, seems also to present a barrier to crystallization. D-Idose, which has significant amounts of the pyranose C1 and 1C conformation present in solution, is only known as a sirup at the present time.

For use in foods, the fact that tautomeric forms of a sugar are impurities in themselves, and inhibit crystallization, is not necessarily a disadvantage. On the contrary, "invert" sugar is preferred for use in certain food products because it is more resistant to crystallization than sucrose sirups, and when handled in bulk, presents less of a crystallization problem.

The reducing sugars can sometimes be crystallized in one or both of the pyranose anomeric forms. Once crystals have been obtained, the process can be repeated without difficulty, even if the solution is not intentionally seeded with crystals. The phenomenon has been ascribed to "nucleating crystals in the environment," and many intriguing and seemingly mysterious tales are told of the crystallization of a sugar for the first time in any one laboratory. Sugars being prepared for the first time in pure form have been known to persist for years in sirupy form, and then for reasons unknown, to crystallize. The distribution of seed crystals to other laboratories readily causes sirups of sugars prepared there to crystallize.

A personal experience of one of the authors is pertinent. After several years of periodic attempts, the tetrasaccharide stachyose was induced to crystallize from a water-acetone solution (Shallenberger 1961). The source of the sugar was tubers of the Japanese artichoke

(*Stachys sieboldii, Stachys tuberifera*) which were sent to a friend who wished to prepare the sugar. Upon preparing pure sirups in two different laboratories, he could not induce either one of them to crystallize. Seed crystals from the original preparation were then forwarded, and when added to one of the sirups, it immediately crystallized. Proceeding to the second laboratory to seed the second preparation, the chemist was startled to find that it too had crystallized! In the laboratories of C. S. Hudson and N. K. Richtmyer, at the National Institutes of Health, Bethesda, Maryland, it is common practice to reduce some of the crystals of a new sugar to a powder and blow them into the atmosphere so that the sugar could easily be crystallized in those laboratories in the future.

There are many reasons why it is desirable to obtain sugars in their crystalline form. Foremost among them is that impurities can be decreased by repeated crystallization, and characteristic melting points and optical properties can be determined. The analysis of crystal structure by X-ray techniques can indicate the configuration—even the absolute configuration—of a compound, its conformation, bond lengths, and the valence angles associated with the intermolecular stereochemistry and structure of its crystal lattice (Jeffrey and Rosenstein 1964).

Sugars form different crystal types not only according to molecular structure, but also according to the way the molecules are packed into the cell of the crystal lattice. They often exist, therefore, in two or more allotropic forms in much the same way that carbon exists as diamond, graphite, or lamp-black. This causes problems in the identification of a sugar by its melting point.

Most sugar crystals have a specific gravity in the region of 1.5; as a result of the strong hydrogen-bond forces in the crystal lattice, they have high melting points, seldom below 100°, and frequently in the range 200 to 300°. A number of sugars are known which do not melt below the decomposition temperature of somewhat more than 300°. During a long, slow heating process, such as is employed in the determination of a melting point, it is frequently observed that the crystal alters its allotropic form. Such changes can best be recorded with modern differential thermal scanning equipment.

Sugars may also crystallize in various hydrated forms that have different solubilities, melting points, etc. The characteristics of crystalline forms of the important food sugars are given below.

α-D-Glucose.—D-Glucose crystallizes from water solution at concentrations between 30% and 70% and temperatures from 0° to 50° as the monohydrate ($C_6H_{12}O_6 \cdot H_2O$) of the α-D-pyranose isomer. Among the known crystalline forms of this glucose monohydrate

is a pentagonal leaflet; after rotation of the pentagonal leaflet by 180°, a twinning of the leaflets occurs, resulting in a monoclinic spheroidal form. Another form resembles spicules grown from a common nucleus, with a structure very much like that of a sea urchin (Newkirk 1936). The melting point of the monohydrate is reported to be 83°, and the optical rotatory power is calculated to be +102° → +47° (Bates 1942).

Anhydrous α-D-glucose is obtained by evaporating glucose solutions at temperatures somewhat above 50°. Anhydrous α-D-glucose crystals bear a superficial relation to hydrated glucose crystals (approximately pentagonal), but are more highly refractive and more compact. They melt at 146°. The optical rotatory data are given in Table 3.4.

TABLE 3.4

SPECIFIC ROTATION OF SUGARS ON AN ANHYDROUS BASIS

Sugar	$[\alpha]_D^{20}$ "Initial"	Final
α-D-Glucose	+112	+52
β-D-Glucose	+19	+52
α-D-Galactose	+144	+80
β-D-Galactose	+52	+80
α-D-Mannose	+34	+15
β-D-Mannose	−17	+15
β-D-Fructopyranose	−133	−92
β-D-Fructofuranose	−21 (−17)	−92
α-D-Xylose	+92	+19
β-D-Xylose	−20	+19
α-D-Lactose	+90	+55
β-D-Lactose	+35	+55
β-D-Maltose	+118	+136
α,α-Trehalose		+178
Sucrose		+66.5
Raffinose		+124
Stachyose		+148

β-D-Glucose.—β-D-Glucose is more easily soluble than the α-anomer, and consequently has greater *initial* solubility. It crystallizes from water solutions in the anhydrous form at temperatures of 100° and above. It also crystallizes from hot acetic acid and from pyridine as a pyridinium complex. The pyridine can be removed from the crystals by heating at 105°. The melting point of β-D-glucose is 148–150°.

β-D-Fructose.—The only known crystalline isomer of D-fructose is β-D-fructopyranose. When prisms of the usual anhydrous form,

TABLE 3.5

INFRARED HYDROXYL ABSORPTION BAND POSITIONS (CM^{-1}) FOR FREE AND HYDROGEN-BONDED OH GROUPS AND THE RELATIVE STRENGTH ($\Delta\nu$) OF THE HYDROGEN BOND FOR SELECTED SUGARS

Sugar	Free OH	Bonded OH	$\Delta\nu$
β-D-Fructose	3520	3400	120
Sucrose	3570	3395	175
Raffinose		3180	390?
α-D-Glucose	3410	3315	95
β-D-Glucose	3545	3340	205
α-D-Galactose	3380	3210	170
α-D-Mannose	3450	3340	210
α-D-Lactose	3530	3360	170
β-D-Lactose	3460	3380	80

which melt at 102° to 104°, are dissolved in water (75% solution) and stored at 0°, seeding with the anhydrous form causes the formation of spherulites of fructose hemihydrate. Upon vigorous stirring, large well-structured prisms of fructose dihydrate form, which melt at +21.3° (Young *et al.* 1952A; 1952B). Since the solubility of the dihydrate decreases rapidly with lower temperatures (it has only $^3/_5$ of the solubility of anhydrous fructose at -9°), and it crystallizes rapidly, even in solutions saturated with glucose, the procedure appears well-suited for the preparation of fructose. Young *et al.* (1952A) constructed a fructose-water phase diagram which indicates that above 21.4°, anhydrous fructose is the stable form. The hemihydrate is stable between 19.9° and 21.4°. The dihydrate is stable at temperatures below 19.9°, and a gel form can exist between -20 and +10°. Anhydrous fructose can also be obtained by crystallization at ambient temperatures from ethanol (Verstraeten 1967).

Fructose was known only as a sirup for many years ("sucre liquide" and the "uncrystallizable sugar"). The difficulty encountered in preparing crystalline fructose for the first time is probably due to the fact that at mutarotational equilibrium, the β-D-pyranose anomer is in equilibrium with significant amounts of the β-D-furanose tautomeric form rather than the α-D-pyranose anomer.

α- and β-D-Galactose.—α-D-Galactose normally crystallizes from water or alcoholic solutions at room temperature as the anhydrous pyranose form, which melts at 165 to 167°. While β-D-glucose can be prepared by crystallization at temperatures above 100°, β-D-galactose crystallizes from an ethanol-water solution at 0°.

When anhydrous β-D-galactopyranose, which melts at 143 to 146°,

is melted and maintained at a temperature of 140°, it resolidifies and then melts again at 164 to 167°. Apparently an equilibrium between the two pyranose anomers is established in the original melt, and as the α-form crystallizes it does so at the expense of the β-form (Wolfrom *et al.* 1954).

α- and β-D-Mannose.—The anhydrous crystalline form of both α-D- and β-D-mannose are known. An oddity in the records of carbohydrate chemistry is the fact that it was the anhydrous and higher-energy form, β-D-mannopyranose, that was first crystallized, and that form became available from chemical supply houses. Hudson and Sawyer (1917) attribute the initial crystallization to Alberda-Van Ekenstein in 1896. It melts at 132° and has a slightly sweet taste, followed by a distinctly bitter taste. When the lower-energy form, α-D-mannopyranose, was crystallized, it became very difficult to obtain the β-D-form in the same laboratory, and α-D-mannose replaced the β-form as the commercially available substance. Its melting point is close to that of the β-D-form (133°), but the two anomers are readily distinguished by their optical rotatory behavior. A method for converting the usual anhydrous α-D-mannopyranose to the β-form is described by Levine *et al.* (1968).

α- and β-D-Lactose.—The usual crystalline form of lactose is the monohydrate of the α-D-anomer. Its crystals are of the monoclinic spheroidal form (sometimes described as "tomahawk-like"), and melt at 201 to 202° after having first given off the water molecule of crystallization at 120°. The crystals are difficult to dissolve, but about 20 gm per 100 ml are soluble in water at room temperature. Fries *et al.* (1971) report that crystalline α-D-lactose monohydrate contains about 7% random distribution of β-D-lactose in the crystal. They also report the presence of an intramolecular hydrogen bond. Since there are no less than 16 hydrogen bonds linking one lactose molecule to its neighbors in the crystal (Beevers and Hansen 1971), the difficulties encountered in dissolving α-D-lactose monohydrate seem related to this fact.

α-D-Lactose monohydrate crystallizes from aqueous solution at ambient temperatures, but anhydrous β-D-lactose, like β-D-glucose, crystallizes from aqueous solution at temperatures above 95° (Hudson 1908). This anomer melts at 252°, and is more easily soluble than the α-anomer.

α- and β-D-Melibiose.—Melibiose occurs in foods in trace amounts whenever the food contains raffinose. It may occur in significant amounts when a yeast invertase is present to hydrolyze the sucrose unit of raffinose. As with mannose, the higher-energy form, β-D-melibiose dihydrate, was obtained first. This compound melts

at 85 to 86°. Subsequently, Fletcher and Diehl (1952) discovered and purified the α-D-monohydrate. It has lower solubility than the β-D-anomer, and consequently has superior crystallizing ability. It melts at 184 to 185°.

Manninotriose.—The reducing trisaccharide manninotriose may occur in foods under the conditions described for melibiose, but the parent sugar is the tetrasaccharide stachyose. Manninotriose can be considered to be a galactosyl melibiose. It was first prepared by Tanret (1902) by acetic acid hydrolysis of the β-D-fructofuranoside moiety of the sucrose component of stachyose. Faintly birefringent spherules were recovered by precipitating with 80% ethanol. These seemed to melt at 150°, and although Onuki (1932) considered manninotriose prepared in this manner to be crystalline, Tanret stated that he was never able to obtain the sugar in a distinctly crystalline form.

Manninotriose, obtained in the laboratories of the N.Y. State Agricultural Experiment Station, was prepared by the action of a *Sacchromyces* species of Champagne yeast on stachyose. It was precipitated from solution with acetone, and the friable powder of spherules obtained was faintly birefringent. They too seemed to melt at 164 to 169° and had a specific rotation of +167° → +149°. Since the product contained 12.8% H_2O it was presumed to be a crystalline tetrahydrate, but the absence of any X-ray powder diffraction pattern proved that the material was not crystalline.

Maltose.—Of the two anomers possible, only β-D-maltose is known as a crystalline substance. It is the monohydrate, and melts at 102 to 103°. Isomaltose (6-*O*-D-glucopyranosyl-D-glucose) and maltotriose are known only as amorphous substances.

Sucrose.—Sucrose crystals have the prismatic habit. This sugar, being a nonreducing disaccharide, is easily crystallized in anhydrous form. The melting point is variable, however, and values of from 160 to 186° have been reported.

In foods, various hydrated forms may crystallize at low temperatures. Sucrose hemipentahydrate (2.5 H_2O) and hemiheptahydrate (3.5 H_2O) form spherulitic aggregates of fine crystals that have a mold-like appearance (Fig. 3.6). These crystalline structures form most readily at -23°, which is close to the temperature (-10°F) used in the commercial storage of frozen foods. Their formation can be minimized by replacing part of the sucrose used in the preparation of the food with invert or another sugar (Young *et al.* 1950).

Raffinose.—The trisaccharide raffinose crystallizes from water solution as the pentahydrate, which melts at 80°. The crystals are difficult to dissolve, however. The specific rotation of raffinose pentahydrate is +100°.

Courtesy of F. T. Jones and F. E. Young, U.S. Dept. Agr.

FIG. 3.6. SUCROSE HYDRATE SPHERULITIC AGGREGATES GROWN FROM SYRUP AT –23°

Stachyose.– Stachyose crystallizes from water-ethanol or water-ethanol-acetone solutions. The crystals have been reported to be either a tetra- or pentahydrate, and the possibility that stachyose crystals are a heminonahydrate ($4.5\ H_2O$) cannot be ignored.

The melting point of stachyose appears to vary with the technique used for determining it. In a sealed tube, the melting point is reported to be 101 to 105° (Wolfrom *et al.* 1952). Crystals of a "tetrahydrate" from water-ethanol-acetone solutions (Shallenberger 1961) melted at 158 to 160° on a hot stage. Tanret (1902) reported that stachyose (manneotetrose $\cdot\ 4.5\ H_2O$) softens at 150° and melts at 167°. The specific rotation of the hydrate is about +131°.

Osmotic Pressure of Sugar Solutions

The phenomenon of osmotic pressure has important application in the field of food sugar chemistry, since sugars in solution have an intrinsic osmotic pressure. If a sugar solution is enclosed in a membrane that will permit the transit of water, but not of sugar (i.e., it is semipermeable), external water molecules will diffuse into the sugar solution and a pressure will be generated against the walls of the membrane that is directly proportionate to the sugar concentration.

Osmotic pressure may be defined as the pressure that must be applied to such a system to prevent the inward diffusion of water, but the term is also used in the abstract sense that a sugar solution has an inherent osmotic pressure, whether a membrane is present or not. Osmosis itself is the diffusion of water molecules from a region of a given vapor pressure to a region that has a "vapor pressure deficit," or from a region of higher water activity to a region of lower water activity.

Since the pressures inherent or developed in sugar solutions are proportionate to the concentration of the sugar, J. H. van't Hoff (1887), of tetrahedral carbon atom fame, suggested that the pressures developed should be analogous to the pressures developed if the solute molecules were considered to be present as a gas (a sucrose gas), and that the classic gas laws of Boyle and Gay-Lussac ($pv = RT$) should apply. Thus, 1 gram molecular weight of sugar, containing Avogadro's 6.023×10^{23} molecules, dissolved in 1 l of solution at standard temperature, should have an intrinsic osmotic pressure, or should be able to exert an osmotic pressure of 22.4 atmospheres.

Due to the fact that sugars in solution form hydrates, which actually lower the water concentration, the pressures developed are somewhat higher than those calculated by applying the gas laws. The results are nearly ideal, as shown in Fig. 3.7, to concentrations of

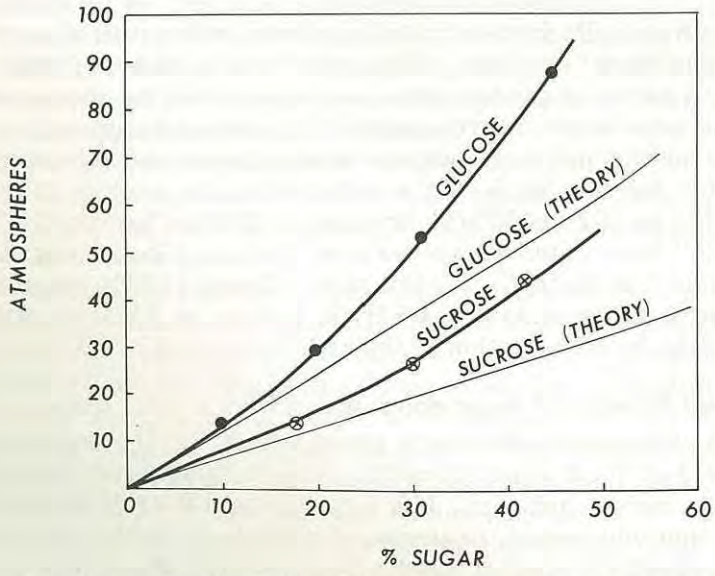


FIG. 3.7. CALCULATED AND OBSERVED OSMOTIC PRESSURES OF GLUCOSE AND SUCROSE SOLUTIONS

about 0.5 molar sugar, and then deviate sharply. Furthermore, the pressures exerted by a monosaccharide are about double those exerted by a disaccharide at the same weight concentration since, at equal percentage concentrations, there are twice as many monosaccharide molecules present in the solution. Sugar solutions have been used to dehydrate fruits and vegetables for preservation.

Water Activity of Sugar Solutions

The ability of the sugars in solution to lower the "water activity" or A_w of the solution, which is related to their osmotic properties, is also an important intrinsic food property. This concept, however, deals directly with the properties of the water in a sugar solution, whereas osmotic pressure is explained on the basis that the sugar molecules themselves behave as a gas.

Water activity, or available moisture, is the vapor pressure of the solution divided by the vapor pressure of water, or

$$A_w = p/p_0$$

where p is the partial pressure of the solution and p_0 is the saturation pressure of water at the specified temperature. The activity of pure water is unity, and for a 1 *molal* solution of a nondissociating substance, it is 0.9823. For example, 1 gram molecular weight of sucrose (342 gm) dissolved in 1 l of water (55.5 gram moles) yields:

$$\frac{55.5}{56.5} = 0.9823$$

The vapor pressure of a solution, then, is equal to the vapor pressure of the pure solvent times the mole fraction of the solvent in solution. The vapor pressure of a solvent is lowered in proportion to its osmotic pressure, and the vapor pressure lowering of water is about 1.77% (100 - 98.23) for a 1 molar ideal solution. Due to hydration of sugar molecules, however, a 1 molal solution has a vapor pressure lowering which is about 10% greater. It follows, therefore, that another method of expressing water activity (Labuza *et al.* 1970) is

$$A_w = \frac{\text{E.R.H.}}{100}$$

where E.R.H. is the equilibrium relative humidity expressed as percentage.

Microbial growth and enzyme activity are related to the water activity of foods (Acker 1969), and nonenzymic browning in dehydrated foods is at an optimum at a given water activity (Labuza *et al.*

1970). The preservative action of sugars in high concentrations is related to the fact that different organisms have different water requirements. The A_w values below which certain bacteria will not grow (Frazier 1958) are:

Pseudomonas	0.96
Achromobacter	0.96
Escherichia coli	0.95
Staphylococcus	0.86
Clostridium botulinum	0.95

Yeasts are classified as "osmophilic" if they grow in an environment with an A_w of about 0.62 to 0.65, while the lower limits for ordinary yeasts are 0.90 to 0.94.

Viscosity

Viscosity (resistance to flow) is an important property of sugar solutions in foods since it imparts thickening and bodying effects.

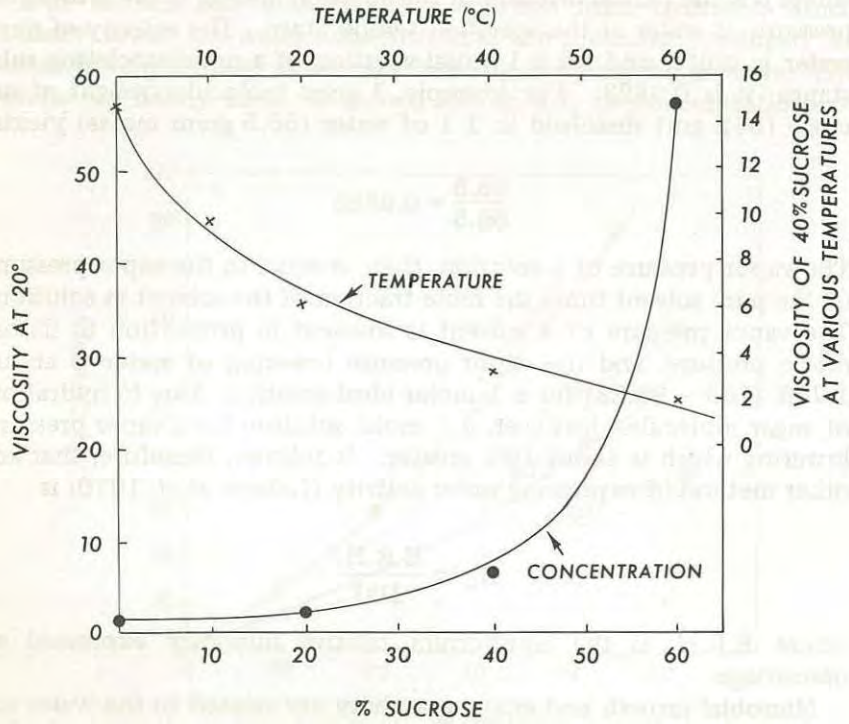


FIG. 3.8. EFFECT OF TEMPERATURE AND CONCENTRATION ON THE VISCOSITY OF SUCROSE SOLUTIONS

The unit for expressing viscosity is the centipoise (0.01 poise). The viscosity of water at 20° is one centipoise, while that of a 60% sucrose solution at 10° is about 100 times as large (one poise). The viscosity of other sugar solutions (fructose, glucose, maltose, and sugar mixtures) is about the same as that of sucrose solutions.

As might be expected by application of hydrogen bond theory, lowering the temperature of a sugar solution increases its viscosity; increasing the sugar concentration obviously increases viscosity, and viscosity increases very rapidly at sugar concentrations greater than 40%. These relations are shown in Fig. 3.8. Tables on the viscosity of sugar solutions at various concentrations and temperatures are given by Bates (1942).

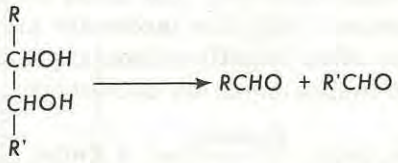
GENERAL CHEMICAL PROPERTIES

The general chemical properties of sugars are the subject of both elementary and advanced works (Guthrie and Honeyman 1968; Pigman 1957; Pigman and Horton 1972; Whistler and Wolfrom 1962) and their reactions have been studied in depth. Authoritative annual reviews are recorded in the *Advances in Carbohydrate Chemistry and Biochemistry* (Academic Press, N.Y.) published annually since 1945.

The intrinsic chemical reactions that are important in foods are discussed in Chapter 4, and some of the fundamental and intrinsic properties are presented in Chapters 1 and 2. General chemical reactions that are of great value in studying the properties and structure of individual sugars and their behavior are now described.

Glycol-splitting Reactions

Lead tetraacetate in glacial acetic acid solution, or sodium metaperiodate in aqueous solution, cleaves the α-glycol grouping of sugars or related polyols (e.g., inositol) to produce carbonyl compounds:



Lead tetraacetate is used for sugar derivatives (e.g. partial esters) that are soluble in nonpolar solvents. Periodate reagents are used for the sugars themselves.

The reaction works for both *cis* and *trans* α-glycol units, but generally cleavage of *cis* glycols proceeds more quickly. If one of the carbon atoms contains a carbonyl or an amino group instead of an

hydroxyl group, the reaction will still take place, but not if the amino group is substituted, as in the case of the acetamido sugars.

Glycol-splitting reactions are used in determining the ring size of sugar molecules. Fructose, for example, can exist either in the 5-membered furanose form, in which it is known as part of the sucrose molecule, or in the 6-membered pyranose form. Since cleavage of each α-glycol grouping results in the consumption of 1 mole of the periodate oxidant, complete oxidation of the pyranose form would require the consumption of 4 moles of periodate. Complete oxidation of the furanose form would require only 3. Since almost 4 moles of periodate are consumed, the sugar exists largely as fructopyranose in solution. Oxidation of secondary alcohol groups results in the release of formic acid, whereas if primary alcohol groups are present, formaldehyde is produced. These reaction products can be used to monitor the course of the reaction and are of value in the analysis of certain polyols, such as sorbitol in foodstuffs. Glycol-splitting reactions may also be used to determine the position of linkage in polysaccharides of known ring size, the principle being very similar to that given.

Condensation Reactions

Aldoses and ketoses react with hydroxylamine, eliminating water of condensation and forming oximes:

$$R\text{-}CHO + H_2NOH \longrightarrow R\text{-}CH = NOH + H_2O$$

and

$$R\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_2OH + H_2NOH \longrightarrow R\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}CH_2OH + H_2O$$

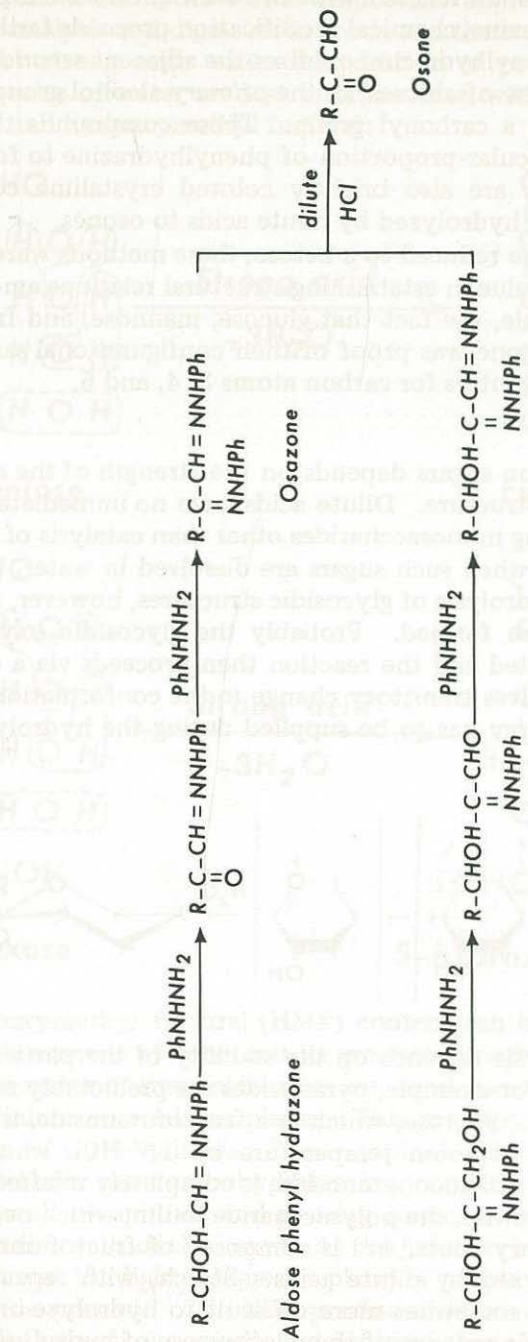
Oximes are often crystalline compounds with well-defined melting points useful for characterizing the sugars, but they are not so well known as the phenylhydrazones. The latter are formed by condensing aldoses and ketoses with one molecular proportion of phenylhydrazine; they are often brightly colored crystalline derivatives that show characteristic shapes under the microscope.

$$R\text{-}CHOH\text{-}CHO \xrightarrow{PhNHNH_2} R\text{-}CHOH\text{-}CH = NNHPh$$

Aldose — *Aldose phenyl hydrazone*

$$R\text{-}CHOH\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH_2OH \xrightarrow{PhNHNH_2} R\text{-}CHOH\text{-}\underset{\underset{NNHPh}{|}}{C}{=}CH_2OH$$

Ketose — *Ketose phenyl hydrazone*

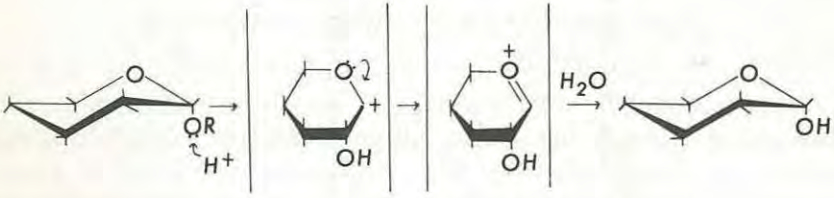

R-CHOH-CH = NNHPh
PhNHNH2
R-C-CH = NNHPh
O
R-C-CH = NNHPh
NNHPh
Aldose phenyl hydrazone
Osazone
dilute
HCl
R-C-CHO
O
Osone
R-CHOH-C-CH2OH
NNHPh
PhNHNH2
R-CHOH-C-CHO
NNHPh
PhNHNH2
R-CHOH-C-CH = NNHPh
NNHPh
Ketose phenyl hydrazone
Osazone

When phenylhydrazones are reacted with two further molecular proportions of phenylhydrazine, chemical modification proceeds farther. The second mole of phenylhydrazine oxidizes the adjacent *secondary* alcohol group in the case of aldoses, or the *primary* alcohol group in the case of ketoses, to a carbonyl group. These compounds then react with a third molecular proportion of phenylhydrazine to form an *osazone*. Osazones are also brightly colored crystalline compounds and they can be hydrolyzed by dilute acids to osones.

Since an osone can be reduced to a ketose, these methods were of considerable historical value in establishing structural relations among the sugars. For example, the fact that glucose, mannose, and fructose gave the same osazone was proof of their configurational sameness at the asymmetric centers for carbon atoms 3, 4, and 5.

Action of Acids

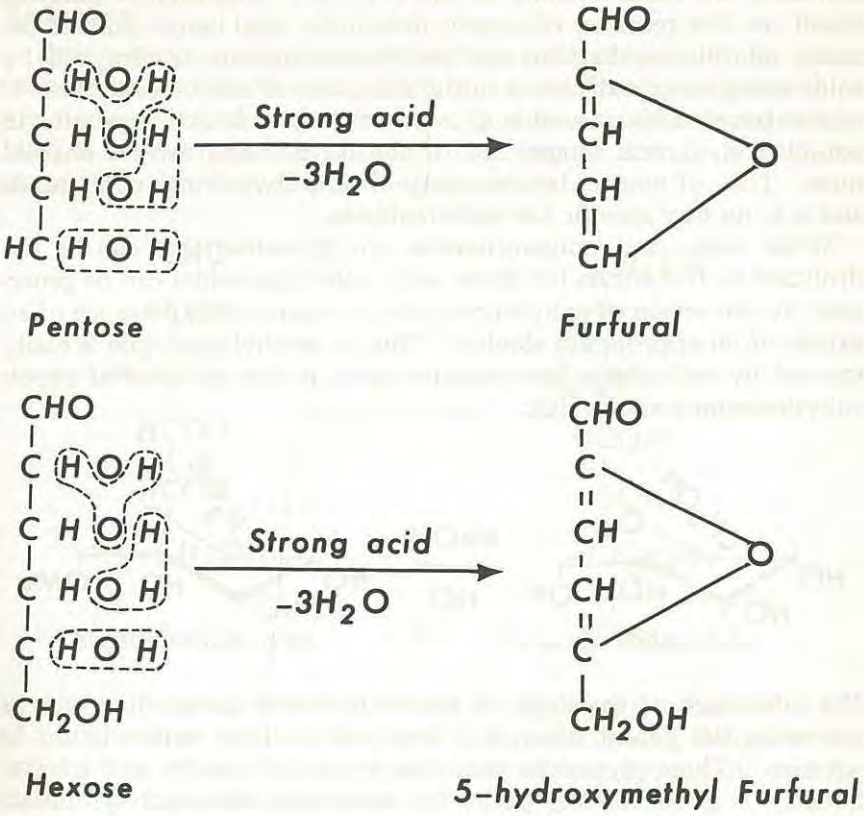
The action of acids on sugars depends on the strength of the acid and the type of sugar structure. Dilute acids have no immediate effect on the free reducing monosaccharides other than catalysis of the mutarotation reaction when such sugars are dissolved in water. Dilute acids can cause hydrolysis of glycosidic structures, however, and free reducing sugars are formed. Probably the glycosidic oxygen atom becomes protonated and the reaction then proceeds via a carbonium ion. This involves transitory change in the conformation of the ring and hence energy has to be supplied during the hydrolysis, usually in the form of heat.



The ease of hydrolysis depends on the stability of the particular glycosidic structure. For example, pyranosides are predictably more stable than furanosides. Sucrose, which is a fructofuranoside, is hydrolyzed very quickly at room temperature by 1*N* HCl, whereas α,α-trehalose, which is a diglucopyranoside, is completely unaffected by 5*N* HCl at 37°. Likewise, the polysaccharide inulin, which occurs in dandelion and chicory roots, and is composed of fructofuranose units, is easily hydrolyzed by dilute acids. Starch, with repeating glucopyranose units, is somewhat more difficult to hydrolyze under the same conditions. Knowledge of the relative ease of hydrolysis of

different carbohydrates has important application with regard to their analysis and digestion in foods and their use in medicine.

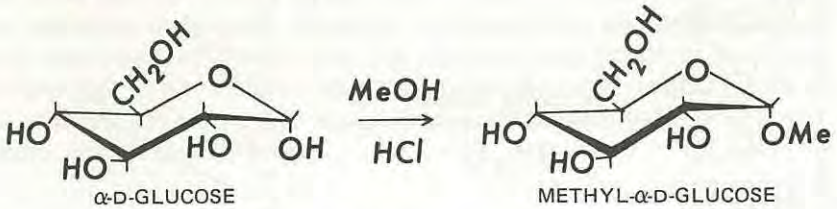
Hot strong acids cause dehydration of pentoses and hexoses; 3 molecules of water are lost in either case, with the formation of furfural and hydroxymethyl furfural:



Hydroxymethyl furfural (HMF) content can be used as an index of heat treatment of food, but its greatest importance (as with furfural) is that either of these aldehydes can condense with amines or phenols to produce colored complexes. This property is used in the identification of carbohydrates. For example, Molisch's test, which is a general test for most carbohydrates, is accomplished by mixing the solution with α-naphthol and pouring concentrated sulfuric acid slowly down the walls of the vessel. At the junction between the heavy acid phase and the lighter aqueous phase, a purple ring forms if carbohydrate is present. The heat produced when the concentrated acid meets the aqueous solution dehydrates the sugar, and the resulting

furfural condenses with the phenol. Amino sugars and many other sugar derivatives that lack hydroxyl groups appropriate for dehydration to furfurals do not give a positive Molish test. Other well-known carbohydrate detection reactions, such as Bial's orcinol test for pentoses, Seliwanoff's test for ketoses, and Tollen's reaction, also for pentoses, are modifications of this principle. Quantitative analyses based on this reaction obviously determine total carbohydrates because all oligosaccharides and polysaccharides are hydrolyzed by acids and consequently react in the same way as monosaccharides. If concentrated sulfuric acid is allowed to dehydrate carbohydrates to completion, it will remove all of the oxygen and leave a charred mass. This, of course, is a property of all polyhydroxy compounds and is in no way specific for carbohydrates.

While oligo- and polysaccharides are glycosides that can be hydrolyzed to free sugars by dilute acids, other glycosides can be generated by the action of anhydrous acids on sugars in the presence of an excess of an appropriate alcohol. Thus, a methyl glycoside is easily formed by refluxing a free reducing sugar in the presence of excess anhydrous methanolic HCl:



α-D-GLUCOSE METHYL-α-D-GLUCOSE

The advantage of this type of glycoside is that the product closely resembles the parent sugar, but does not undergo mutarotation in solution. These glycosides therefore crystallize easily, and are frequently a good starting point for multistage chemical syntheses. Other general reactions of sugars with acids, with special emphasis on food systems are discussed in Chapter 4.

Action of Alkalies

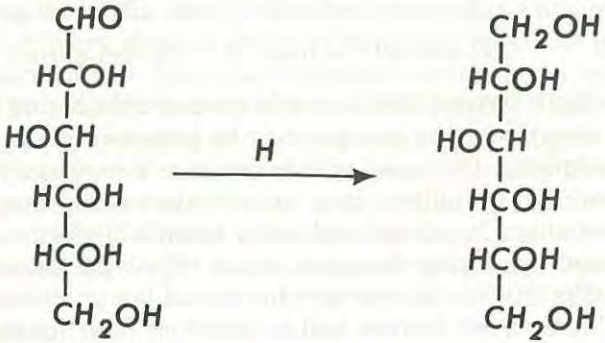
Alkalies cause the destruction of reducing sugars, but are without effect on glycosides. They are also without effect on ethers and acetals of sugars, but they do react with sugar esters to remove the ester groupings (saponification) to generate the free sugar. Under anhydrous conditions a trace of alkali can cause catalytic removal of ester groupings without consumption of alkali. Under aqueous conditions, however, consumption of alkali depends on the equivalent number of ester groupings in the sugar derivative, and can therefore be used to

assist in the determination of the structure of partially esterified sugar molecules.

Other actions of alkali on sugars, such as catalysis of mutarotation, enolization and isomerization of the sugars, and the formation of saccharinic acids are reserved for Chapter 4.

Reduction

Free sugars can be reduced by sodium amalgam and lithium aluminum hydride either electrolytically or by catalytic hydrogenation to yield sugar alcohols. For example, glucose can be reduced to sorbitol, a sweet crystalline substance used largely in diabetic foods because of its slow rate of intestinal absorption.



Glucose **Sorbitol (Glucitol)**

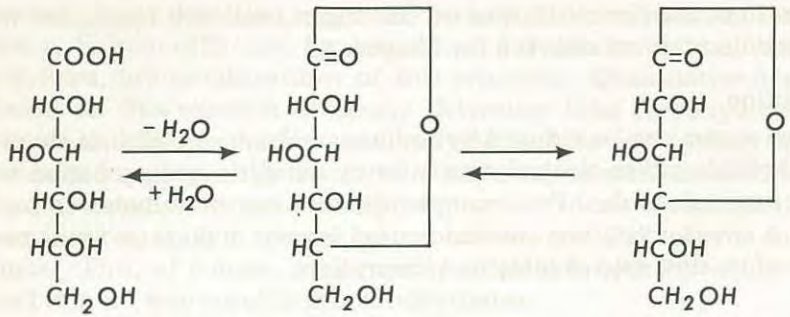
In general, sugar alcohols are named by placing the suffix *-itol* at the end of the stem name of the parent sugar. Sorbitol is, therefore, the trivial name of a substance which, according to systematic nomenclature, should be called glucitol.

When ketoses are hydrogenated, a new asymmetric carbon atom is formed and two products result. Fructose is thus converted to a mixture of sorbitol and mannitol, the latter also being a substance sometimes used in diabetic foods. The corresponding alcohol of the sugar galactose is galactitol, another crystalline sweet substance with the trivial name dulcitol. It is not used for food purposes because it has been implicated in cataract formation when galactose is fed to rats.

Oxidation

Aldoses, but not ketoses, are oxidized by mild reducing agents, such as bromine water or sodium hypoiodite, to aldonic acids. These hydroxy acids may lose an intramolecular molecule of

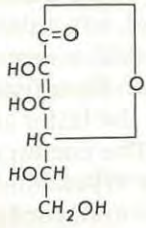
water in solution to form lactones, and an equilibrium is established between the free acid and the lactones possible:



Gluconic acid *Glucono-delta-lactone* *Glucono-gamma-lactone*

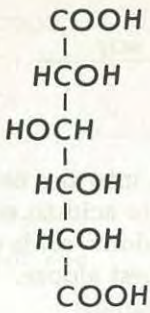
Usually it is the γ-lactone that is stable (exo-double bonds stabilize 5-membered rings), and the lactone may be obtained by evaporating an aqueous solution. Gluconic acid finds use as a sequestering agent for metals, and for solubilizing iron and calcium so they may be presented to the human in neutral and easily assimilable form. Aldonic acids are named by taking the stem name of the parent sugar and adding the suffix *-onic*. Aldonic acid formation is a good way of distinguishing aldoses from ketoses and is therefore used for determining aldoses and ketoses in the presence of each other.

Vitamin C (L-ascorbic acid) is a lactone of an unsaturated derivative of gulonic acid. Based on carbohydrate nomenclature rules, it is 2-keto-L-threo-hexano-γ-lactone-2,3-enediol. It is synthesized in quantity for use in foods and medicine.



L-Ascorbic acid (Vitamin C)

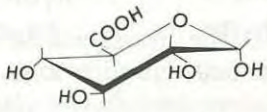
Vigorous oxidation of reducing sugars, for example, in the presence of nitric acid, results in oxidation of the primary alcohol group as well as the carbonyl group, to form a dicarboxylic acid. Glucose yields glucaric acid.



Glucaric acid

The dicarboxylic acids are called "aldaric" acids and are named by appending the suffix *-aric* to the stem name of the parent sugar.

If the primary alcohol group of the sugar molecule is oxidized in such a manner as to leave the hemiacetal carbon atom hydroxyl unaffected, the product is known as an uronic acid. Glucose yields glucuronic acid, so-named by adding the suffix *-uronic* to the stem name. This type of acid cannot be formed directly from free reducing sugars because the potential carbonyl group is so reactive that it is oxidized first. Uronic acids are therefore usually prepared from natural sources. They may be prepared however by oxidation of glycosides since the hemiacetal carbon atom hydroxyl is protected from oxidation:

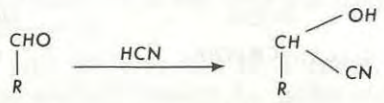


Glucuronic acid

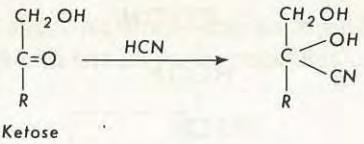
The important food polysaccharide, pectin, consists of chains of galacturonic acid residues, some of which have the C-6 acid group esterified with methyl groups. Because free reducing sugars are susceptible to oxidation by a wide variety of oxidizing agents, they sometimes act as useful antioxidants themselves.

Cyanohydrin Reaction

Both aldoses and ketoses react with HCN to form cyanohydrin addition compounds:

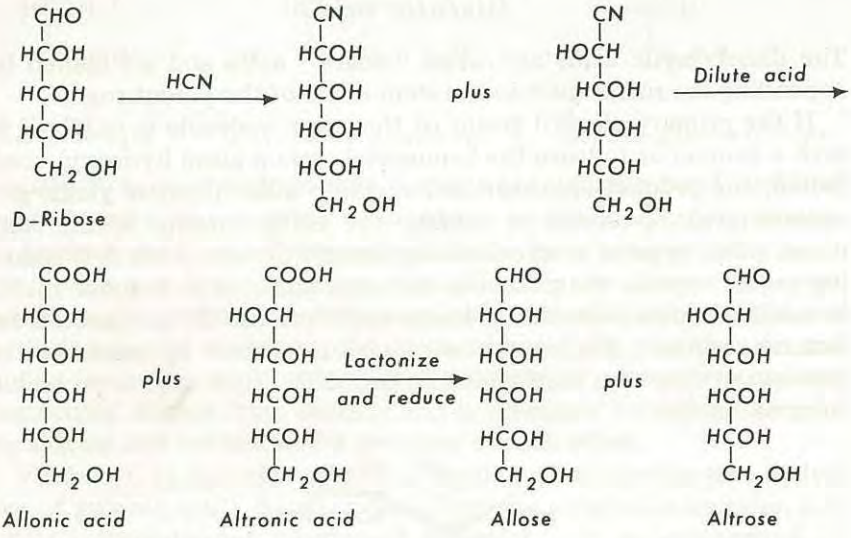


Aldose



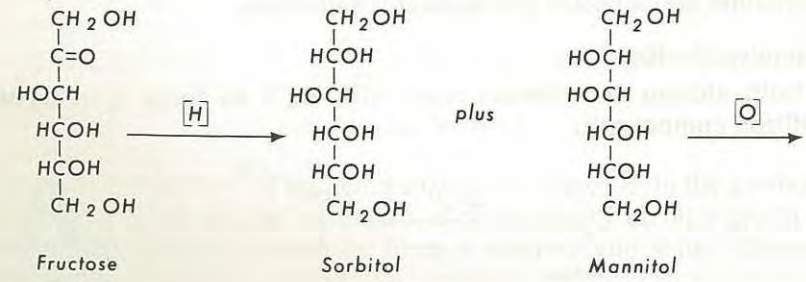
Ketose

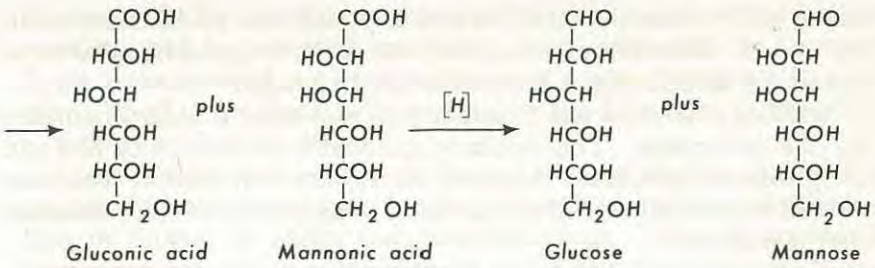
When an aldose is converted into the corresponding cyanohydrin, it can be hydrolyzed with dilute acid to convert the cyano group to a carboxylic acid. The two aldonic acids can be separated, lactonized, and reduced to the next highest aldose. Thus, starting with D-ribose, allose and altrose can be prepared:



Conversion of Ketoses to Aldoses

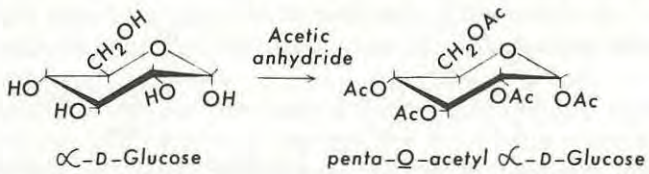
A ketose can be reduced to a mixture of the two corresponding sugar alcohols. These are then oxidized chiefly to the aldonic acids which, upon being lactonized, can be cautiously reduced to the corresponding aldoses:





Esterification

Since all sugars are polyhydroxy compounds, they can be esterified at all free hydroxyl positions by acids and anhydrides. The choice of solvent and catalyst may govern the anomeric form of the product. The most popular method of generating an acetylated sugar with a specific anomeric configuration involves dissolving the sugar in pyridine and acetic anhydride and allowing the reaction to proceed at room temperature for several hours. The original configuration at the hemiacetal carbon atom is retained in the acetylated derivative:



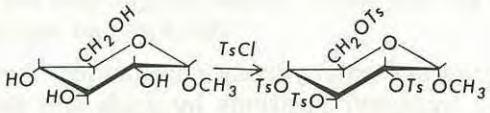
α-D-Glucose can also be acetylated with acetic anhydride and zinc chloride as a catalyst, and although the reaction requires heat, it again yields predominately α-D-glucose pentaacetate. If zinc chloride is replaced with sodium acetate as catalyst, the β-acetate is favored. The β-pentaacetate, on being heated with zinc chloride, will again be converted to the α-form.

Benzoate esters are formed by dissolving the sugar in pyridine containing benzoyl chloride, and shaking. Benzoate formation is as facile as sugar acetylation, but benzoate esters have the advantage of being more stable. Partially esterified sugar benzoate groups are also less likely to exhibit the phenomenon of "acyl migration," i.e., movement of an ester grouping from one position to another in the sugar ring. Benzoates frequently cause greater chemical shifts in their nuclear magnetic resonance spectra, and this facilitates interpretation of their molecular structure.

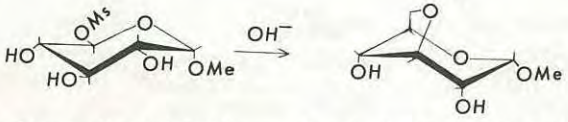
Both benzoates and acetates can be formed quantitatively from their parent sugars, and can easily be isolated from their reaction mixtures by pouring the mixture into cold water. The products crys-

tallize easily because of their insolubility, and have well-defined melting points. Like other esters, they can be hydrolyzed by alkalies, as previously mentioned, to regenerate the free sugar.

Another class of esters frequently encountered in sugar chemistry are the sulfonates. The methane sulfonates (mesylates of Ms) and the *p*-toluene sulfonates (tosylates or Ts) are well known. The reaction, starting with α-methyl glucoside in pyridine and tosyl chloride, is shown below:

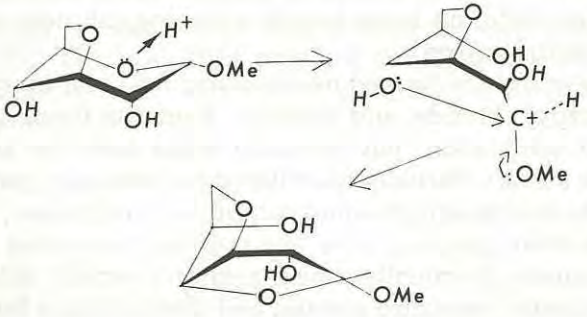


The sulfonate esters are formed just as readily as the acyl esters, but when treated with alkalies they may split at the carbon-oxygen bond of the sugar ring, causing inversion of configuration. Furthermore, alkaline hydrolysis of sulfonates frequently causes anhydro sugar formation; formation of the 3,6-anhydro sugar or the 2,3 epoxide is favored, as shown below:



Methyl-6-O-Ms-α-D-Glucoside *3,6-anhydro α-D-methylglucoside*

The anhydro pyranose rings are remarkably stable to acids and alkalies, but under conditions of acid treatment, they are converted into anhydro furano-rings that are extremely stable (Birch *et al.* 1971):



Etherification

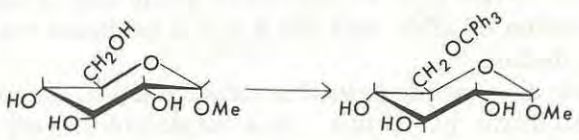
Because the sugars are polyols they react with other alcohols, in the presence of a dehydrating agent, to form ethers. The reaction

does not initially give quantitative yields as easily as the esterification reaction, but by repeated treatment, good yields can be achieved. Typical examples of reagents for this reaction are dialkyl sulfates and alkali, or alkyl iodide and silver oxide for the formation of the corresponding per-*O*-alkyl ether.

The sugar ethers are stable to both acids and alkalies and for this reason are used as a means for discovering the position of substitution or linkage in sugars and polysaccharides. Thus, if maltose is permethylated and then hydrolyzed by an acid, the products found are 1,2,3,6-tetra-*O*-methyl-α-D-glucose and 2,3,4,6-tetra-*O*-methyl-α-D-glucose, indicating that the mode of linkage in the disaccharide is 1 to 4.

Of particular importance are the triphenylmethyl (trityl) ethers. When triphenylmethyl chloride is reacted with any sugar, it preferentially attacks the primary alcohol group because of its steric projection from the plane of the ring. Thus the major product, which can often be isolated in crystalline form, is the selectively substituted 6-*O*-trityl sugar. After blocking the 6-position of hexopyranoses in this way, chemical modification of the secondary alcohol groups of the sugar structure may be carried out, after which the trityl group may easily be removed by the action of acids leaving the 6-position unsubstituted.

Normally the 6-position would react more quickly than the other positions of the sugar ring (except for the highly reactive anomeric center) because of the higher reactivity of the primary alcohol group compared to the secondary alcohol groups. Therefore, this reaction is one of the few means available for modifying secondary alcohol groups in the sugar ring without affecting the primary alcohol group. Furthermore, the unsubstituted 6-position can now be modified, after which the secondary alcohol groups may be desubstituted leaving the original sugar selectively modified in the 6-position:



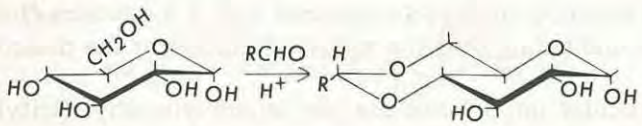
Methyl α-D-Glucoside

Methyl 6-O-triphenyl-methyl α-D-Glucoside

Acetal Formation

Normally, when an aldehyde reacts with an alcohol the product is first a hemiacetal and then an acetal. In the sugars, both alcohol and

aldehyde groups are present in the same molecule, and the aldehyde group reacts intramolecularly with one of the alcohol groups to form a cyclic hemiacetal structure in either the pyranose or furanose form. However, each sugar ring now contains several other alcoholic groups which may react with aldehydes to form acetals. In the case of the glucopyranose ring, the fourth and sixth hydroxyl groups are sterically disposed so that they react together with a single aldehyde molecule to form a new cyclic acetal:



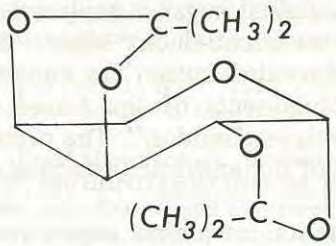
The highly reactive anomeric center causes slight interference with this reaction, so that quantitative yields are not easily obtained. In the case of the glycosides this difficulty does not arise, however, so that methyl α-D-glucopyranoside gives a 100% yield of a crystalline 4,6-benzylidine derivative. All acetals are named by adding the suffix *-ylidene* to the stem name of the alkyl or aryl aldehyde radical from which the acetal was formed. Thus, benzaldehyde gives benzylidene derivatives, and acetaldehyde, which corresponds to the 2-carbon ethyl radical, gives rise to ethylidene derivatives.

Although acetals are formed with the aid of acid catalysts under anhydrous conditions, they are sensitive to acids in the presence of water, reforming the aldehyde and the free sugar. They are very stable to alkalies, however. This means that they behave oppositely to the sugar esters, which are stable to acids, and sensitive to alkalies. Hence it is possible to modify the hydroxyl groups in other than the 4 and 6 positions of glucopyranoside structures by first forming the 4,6-alkylidene derivative and then modifying the remaining part of the molecule. After this, the alkylidene group may be selectively removed by action of acids, and the 4 and 6 positions may be further modified if desired.

Sugar acetals constitute an important class of compounds with valuable surfactant properties. The alkylidene moiety imparts an element of lipid solubility to the molecule, whereas the remaining free hydroxyl groups in the 2 and 3 positions are hydrophilic. Such molecules have important roles in foods as emulsifiers, stabilizers, and flavor media. 4,6-Benzylidene methyl-α-D-glucoside is a cherry flavor development material. Evidently the benzaldehyde is slowly released in foods and gives rise to this flavor characteristic.

Ketal Formation

Ketones react with sugars under anhydrous conditions in the presence of acid catalysts in a similar way to aldehydes. However, in this case the products are ketals and frequently involve different hydroxyl groups in the structure. Reaction with sugar structures with a pair of *cis* hydroxyl groups on adjacent carbon atoms is favored. For example, acetone reacts with galactose with *cis* hydroxyl groups at positions 1 and 2 and 3 and 4 to give 1,2:5,6-di-*O*-isopropylidene-α-D-galactopyranose, while glucose yields 1,2:5,6-di-*O*-isopropylidene glucofuranose, since this is the only glucose structure possible with a pair of *cis* hydroxyl groups:

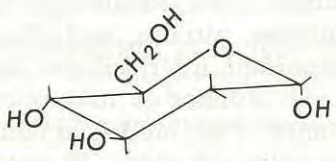


1,2:3,4-*di*-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE

Ketals are similar to acetals in that they are relatively sensitive to acids, releasing the ketone and the free sugar.

Deoxy Sugars

When one of the oxygen atoms of the alcoholic groups of a sugar is missing, the product is called a *deoxy* sugar. Thus, 2-deoxy-α-D-glucose has the structure:



2-DEOXY-α-D-GLUCOSE

Obviously, 2-deoxy glucose and 2-deoxy mannose are the same compound, a situation which arises when other deoxy sugars are prepared from diastereoisomers. Deoxy derivatives of sugars are useful sugar analogs for biological work, e.g., in helping decide which particular hydroxyl groups constitute binding sites for interaction with membranes or enzymes. Unfortunately, the preparation of such com-

pounds is not possible in one step from a given sugar, and in fact requires several stages of sequential modification. After blocking selected hydroxyl groups by acetal and/or ester formation, the desired hydroxyl group may be converted to a sulfonyl ester. This group can then be converted to the deoxy-iodo derivative by replacement of the sulfonoxy moiety with iodine. The deoxy-iodo derivative is usually conveniently reduced with hydrogen and Raney nickel catalyst to the corresponding deoxy compound.

Amino Sugars

When one of the hydroxyl groups of a sugar is replaced by an amino group, the product is an amino sugar. Since the entire hydroxyl group of the original sugar is replaced, the product is more properly described as an amino-deoxy sugar. 2-Amino-2-deoxy glucose and galactose (chrondrosamine) are known to occur naturally, and are structural components of the broad group of substances known as the "mucopolysaccharides." The occurrence of amino sugars in foods as a result of nonenzymic browning reactions is discussed in Chapter 7.

The chemical formation of amino sugars requires several stages. As with the formation of deoxy sugars, a suitably blocked sulfonyl ester is first formed and the sulfonoxy group of this compound is then replaced with an azide group. Reduction of the azide group with hydrogen and Raney nickel then results in the formation of the desired amino sugars. As several of the antibiotics contain amino deoxy sugars, the synthesis of these derivatives is therefore of considerable biological importance.

Oxy-esters

Apart from the sulfonyl esters, sugars may form other oxy-esters such as carbonates, sulfates, nitrates, and phosphates. Carbonates have been used as important intermediates in the preparation of furanoside structures, but sulfates do not appear to have proved of much synthetic importance. They are found naturally as constituents of many sugar units in polysaccharides. Phosphates are of great fundamental importance because of their role in biological energy transfer and in the genetic mechanism. They can be synthesized by the action of phosphoryl chloride, and this reaction is currently employed for several purposes in the food industry.

BIBLIOGRAPHY

ACKER, L. W. 1969. Water activity and enzyme activity. Food Technol. *23*, 1257-1270.

BARKER, S. A., BOURNE, E. J., STACEY, M., and WHIFFEN, D. H. 1954. Infra-red spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. J. Chem. Soc. 171–176.

BATES, F. J. 1942. Polarimetry, Saccharimetry and the Sugars. Circular C. *440*, Nat. Bur. Standards, U.S. Govt. Printing Office, Washington, D.C.

BEEVERS, C. A., and HANSEN, H. N. 1971. Structure of α-lactose monohydrate. Acta Crystallogr. Sect. B. *27*, 1323–1325.

BIRCH, G. G. 1972. Lactose: One of Nature's paradoxes. J. Milk Fd. Technol. *35*, 32–34.

BIRCH, G. G., ETHERIDGE, I. J., and GREEN, L. F. 1972. Short term effects of feeding glucose syrups and their fractions to rats. Brit. J. Nutr. (In press).

BIRCH, G. G., LEE, C. K., and RICHARDSON, A. C. R. 1971. Chemical modification of trehalose. VII. Carbohyd. Res. *19*, 119–122.

BREWSTER, J. H. 1959. A useful model of optical activity. J. Am. Chem. Soc. *81*, 5475–5482.

BROWN, C. A., and ZERBAN, F. W. 1941. Physical and Chemical Methods of Sugar Analysis. John Wiley and Sons, Inc., New York.

ELIEL, E. 1962. Stereochemistry of Carbon Compounds. McGraw-Hill Book Co., Inc., New York.

FLETCHER, JR. H. G., and DIEHL, H. W. 1952. Improvements in the preparation of melibiose from raffinose. A new form of melibiose. J. Am. Chem. Soc. *74*, 5774–5776.

FRAZIER, W. C. 1958. Food Microbiology. McGraw-Hill Book Co., New York.

FRIES, D. C., KAO, S. T., and SUNDARALINGAM, M. 1971. Structural chemistry of carbohydrates. III. Crystal and molecular structure of 4-*O*-β-D-galactopyranosyl-α-D-glucopyranose monohydrate. Acta Crystallogr. Sect. B, *27*, 994–1005.

GUTHRIE, R. D., and HONEYMAN, J. 1968. An Introduction to the Chemistry of Carbohydrates. Clarendon Press, Oxford.

HUDSON, C. S. 1908. Further studies on the forms of milk sugar. J. Am. Chem. Soc. *30*, 1767–1783.

HUDSON, C. S. 1909. The significance of certain numerical relations in the sugar group. J. Am. Chem. Soc. *31*, 66–86.

HUDSON, C. S., and SAWYER, H. L. 1917. The preparation of pure crystalline mannose and a study of its mutarotation. J. Am. Chem. Soc. *39*, 470–478.

ISBELL, H. S., and PIGMAN, W. W. 1937. Bromine oxidation and mutarotation measurements of the alpha- and beta- aldoses. J. Res. Nat. Bur. Stds. *18*, 141–194.

JAFFE, H. H., and ORCHIN, M. 1965. Symmetry in Chemistry. John Wiley and Sons, Inc., New York.

JEFFREY, G. A., and ROSENSTEIN, R. D. 1964. Crystal-structure analysis in carbohydrate chemistry. Advances in Carbohydrate Chem. *19*, 7–22.

LABUZA, T. P., TANNENBAUM, S. R., and KAREL, M. 1970. Water content and stability of low-moisture and intermediate-moisture foods. Food Technol. *24*, 543–550.

LEE, C. Y., SHALLENBERGER, R. S., and VITTUM, M. T. 1970. Free sugars in fruits and vegetables. New York's Food and Life Sciences Bull. *1*. Food Sciences. Cornell University, Geneva, N.Y.

LEVINE, S., HANSEN, R. G., and SELL, H. M. 1968. Preparation of β-D-mannopyranose and some derivatives. Carbohyd. Res. *6*, 382–384.

LOWRY, T. M. 1935. Optical Rotatory Power. Longmans, Green, and Co., London.

MURSCHAUSER, H. 1923. The influence of some normal salts on the mutarotation and specific rotation of glucose. Biochem. Z. *136*, 66–76.

NEWKIRK, W. B. 1936. Development and production of anhydrous dextrose. Ind. Eng. Chem. *28*, 764–766.

ONUKI, M. 1932. Constitution of stachyose. Proc. Imp. Acad. (Tokyo) *8*, 496-499.
PIGMAN, W. W. (Editor) 1957. The Carbohydrates. Academic Press, New York.
PIGMAN, W. W., and HORTON, D. (Editors) 1972. The Carbohydrates. Academic Press, New York.
SHALLENBERGER, R. S. 1961. Preparation of crystalline stachyose. Chem. and Ind. (London) 475.
SHALLENBERGER, R. S. 1963. Hydrogen bonding and the varying sweetness of the sugars. J. Food Sci. *28*, 584-589.
SHALLENBERGER, R. S. 1973. Sugar structure and taste. *In* Carbohydrates in Solution, R. F. Gould (Editor). Advances in Chem. Series *117*, 256-263.
TANRET, C. 1902. On two new sugars derived from manna, namely manneotetrose and manninotriose. Comp. Rend. *134*, 1586-1589.
TOMODA, Y., and TAGUCHI, T. 1930. Effects of sodium bisulfite upon the polarizing power of sugars and the use of sodium bisulfite in sugar analysis. J. Soc. Chem. Ind. (Japan) *33*, 434-441.
VAN'T HOFF, J. H. 1887. The roll of osmotic pressure and an analogy between solutions and gases. Z. Phys. Chem. *1*, 481-505.
VERSTRAETEN, L. M. J. 1967. D-Fructose and its derivatives. Advances in Carbohydrate Chem. *22*, 229-305.
WHISTLER, R. L., and WOLFROM, M. L. 1962. Methods in Carbohydrate Chemistry. Academic Press, New York.
WHIFFEN, D. H. 1956. Optical rotation and geometric structure. Chem. and Ind. (London) 964-968.
WOLFROM, M. L., BURRELL, R. C., THOMPSON, A., and FURST, S. S. 1952. Improved preparation of stachyose. J. Am. Chem. Soc. *74*, 6299.
WOLFROM, M. L., SCHLAMOWITZ, M., and THOMPSON, A. 1954. Observations on the crystalline forms of galactose. J. Am. Chem. Soc. *76*, 1189-1199.
YOUNG, F. E., JONES, F. T., and LEWIS, H. J. 1950. Prevention of growth of sucrose hydrates in sucrose sirups. Food Res. *16*, 20-29.
YOUNG, F. E., JONES, F. T., and LEWIS, H. J. 1952A. D-Fructose-water phase diagram. J. Phys. Chem. *56*, 1093-1096.
YOUNG, F. E., JONES, F. T., and LEWIS, H. J. 1952B. D-Fructose dihydrate. J. Phys. Chem. *56*, 738-739.

Intrinsic Chemical Reactions of the Sugars

When a crystalline sugar is dissolved in a solvent, a series of reactions takes place. The extent to which the reactions occur and their significance depend upon the polarity of the solvent, the pH, temperature, sugar concentration and the time elapsed after dissolution. The intrinsic reactions are mutarotation, enolization and isomerization, dehydration and fragmentation, anhydride formation, and polymerization. These phenomena, in part, are shown in Fig. 4.1 using α-D-glucopyranose as the starting material. The sugars exhibit an amazing degree of versatility in these intrinsic reactions. The equilibria shown are to be viewed as existing to greater or lesser extent under any one set of environmental conditions. However, in different environments, such as higher pH, or altered temperature, identical reaction products may be obtained by an entirely different route, or even mechanism. This seems to be particularly true of the mutarotation reaction and the formation of saccharinic acids and furaldehydes.

The initial reaction shown upon the dissolution of a reducing sugar is *mutarotation*, illustrated in Fig. 4.1 as the establishment of an equilibrium between α-D-glucopyranose (1), aldehydo-D-glucose (5), and β-D-glucopyranose (2). Other isomers are also presumed to be present, and this will be discussed in detail later in this chapter. The mutarotation reaction is named for the fact that the reducing sugars have an optical rotatory power which, when observed in a solution using a polarimeter, changes over a period of time to give a constant value (Lat. *mutare*, to change).

The formation of an enediol (6) from aldehydo-D-glucose is known as the *enolization* reaction. Once formed, the enediol can rearrange in three ways. The starting material, D-glucopyranose, can be regenerated; after a period of time, the ketose β-D-fructopyranose (11) can be formed from keto-D-fructose (10), and α-D-mannopyranose (9) can be generated from aldehydo-D-mannose (8). The structures for the generated pyranoid sugars shown are the favored ones, but are to be viewed as being in equilibrium with their anomers and with all possible isomeric ring forms of the same sugar. The generation of fructose is one case of structural (functional group) isomerism in the sugar series, and the generation of mannose is a special form of structural isomerism known as epimerization.

The formation of saccharinic acids and 5-(hydroxy)-methylfur-

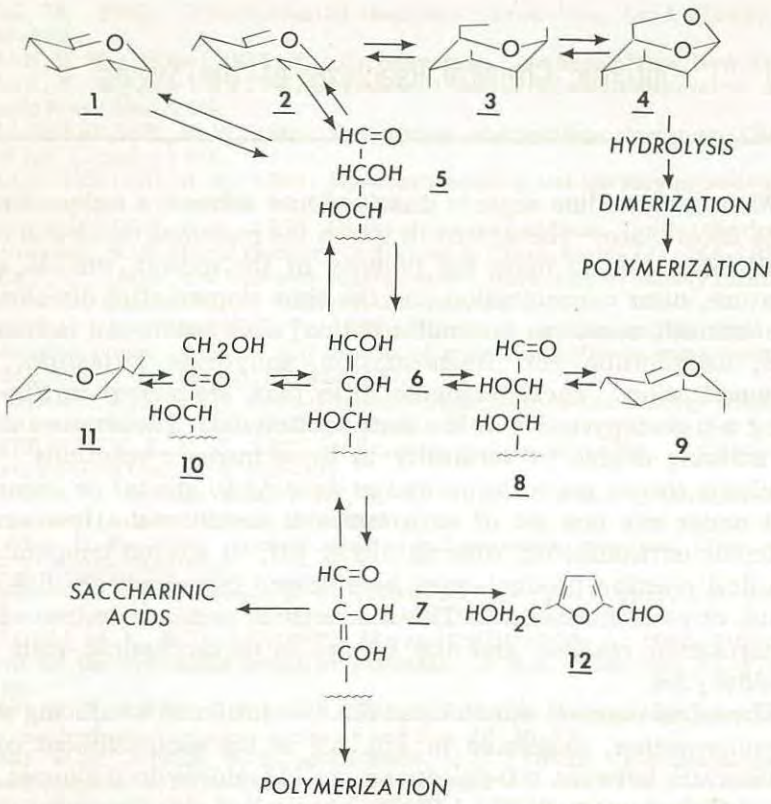


FIG. 4.1. REACTIONS OF REDUCING SUGARS IN SOLUTION

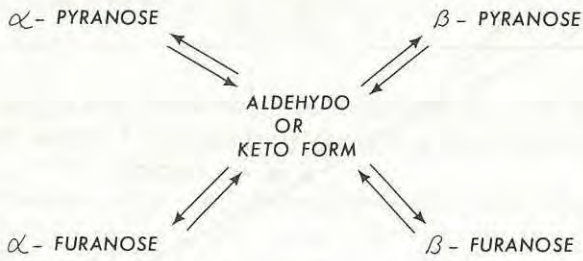
fural (12) from yet another enediol form of aldehydo-D-glucose are examples of possible dehydration and fragmentation reactions. The formation of the anhydro sugar levoglucosan (4) from the alternate 1C conformation of β-D-glucopyranose (3) is also a dehydration reaction. Subsequently, dehydration and fragmentation products of the sugars polymerize to generate materials of higher molecular weight (glucans). When these possess yellow to brown color, and are accompanied by flavorful and tasteful degradation products, they are known as caramels.

All the intrinsic reactions shown occur to a lesser or greater extent over the entire pH scale, but mutarotation, enolization, and the formation of the saccharinic acids are greatly accelerated by an alkaline pH. The formation of sugar anhydrides and furaldehydes is accelerated by an acid pH.

MUTAROTATION

Distribution of Sugar Isomers at Mutarotational Equilibrium

The working hypothesis currently used to explain the mutarotation of reducing sugars in solution assumes that 5 structural isomers are possible for any given reducing sugar. Pyranose and furanose ring forms are believed to be generated from a central acyclic intermediate:



While the concept of a lone intermediate leads to a working hypothesis for the mutarotation phenomenon, several "intermediates" are required to explain known facts about the reaction. These are discussed under the heading of the mutarotation mechanism.

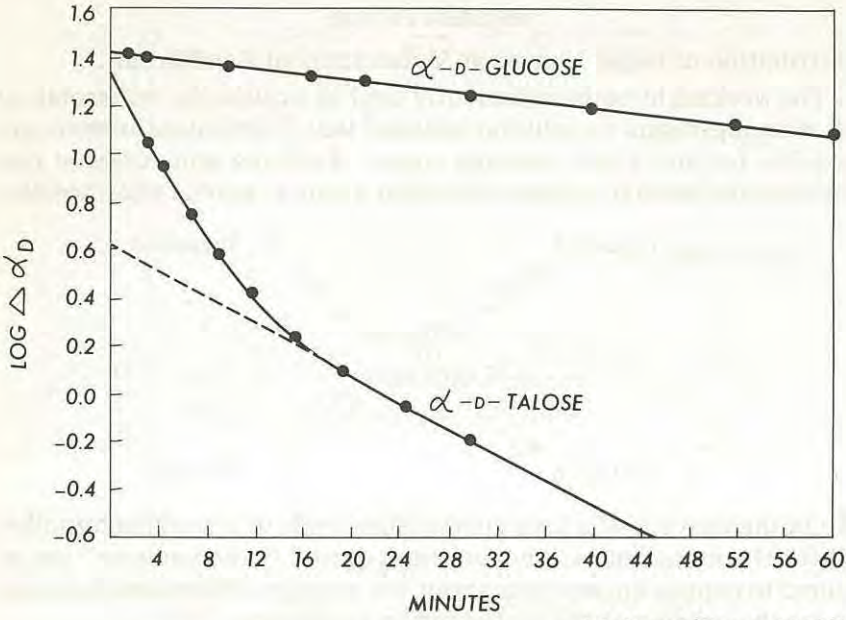
Depending upon the number of isomeric forms present in measurable amounts at equilibrium, a given sugar is said to exhibit "simple" or "complex" mutarotation. Because of the instability of the furanose forms, and the very low concentration of the acyclic modification, D-glucose mutarotates in water solution to give a mixture of about 36% α-D-glucopyranose and 64% β-D-glucopyranose. This is an example of simple mutarotation.

Simple mutarotation is further characterized by uniform values for k_1 and k_2 in the equilibrium expression:

$$\alpha\text{-D-GLUCOPYRANOSE} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta\text{-D-GLUCOPYRANOSE}$$

If the logarithms of the initial optical rotation minus the equilibrium rotation ($r - r_\infty$) are plotted against time, a linear plot is obtained, as shown for D-glucose in Fig. 4.2. Continuous linear plots of this order indicate that there are not more than two forms of the sugar present at equilibrium that are present at a concentration measurable by the technique used to monitor the reaction.

If the values for k_1 and k_2 are not constant, multiphasic plots of $r - r_\infty$ for various times are obtained as shown for α-D-talose in Fig. 4.2. Such plots suggest that more than two forms of the sugar are



Isbell and Pigman (1938)

FIG. 4.2. SIMPLE AND COMPLEX MUTAROTATION CURVES

present at equilibrium. Aldoses that do not have the *gluco*, *manno*, *gulo*, and *allo* configurations exhibit complex mutarotation, and the equilibrium mixture contains at least α- and β-pyranose forms and either the α- or β-furanose form, or both.

Tipson and Isbell (1962) recognize two other types of mutarotation. Certain 2-ketoses, such as D-tagatose, exhibit little or no mutarotation. Other sugars, such as D-fructose, form an equilibrium that consists mainly of a single pyranose form and α- and β-furanose forms. When a sugar shows two rates for the mutarotation reaction, the rapid phase corresponds to furanose formation from pyranoses, or vice versa, and the slow phase corresponds to pyranose-pyranose interconversion (Isbell and Pigman 1938).

The composition of mutarotated sugars in solution has been studied by various chemical and instrumental techniques. Calculation of the pyranose content of solutions from the conformational free energies of pyranose conformational isomers is in good agreement with results obtained by bromine oxidation, optical rotatory data, and nuclear magnetic resonance spectroscopy (Angyal 1968). The latter technique (Angyal 1968), along with gas chromatography of trimethylsilyl ether derivatives of the sugars (Sweeley *et al.* 1963), has been used (Acree *et al.* 1969) to determine the furanose content of

TABLE 4.1

PERCENTAGE DISTRIBUTION OF ISOMERS OF MUTAROTATED SUGARS AT 20°

Sugar	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Glucose	31.1–37.4	64.0–67.9		
D-Galactose	29.6–35.0	63.9–70.4	1.0	3.1
D-Mannose	64.0–68.9	31.1–36.0		
D-Fructose	4.0?	68.4–76.0		28.0–31.6

some of the sugars that show complex mutarotation. The distribution of isomers of some of the naturally occurring food sugars is given in Table 4.1.

The distribution of α- and β-D-anomers for the reducing oligosaccharides lactose and maltose is about the same as that for glucose, or about 32% α- and 64% β-D-anomer (Isbell and Pigman 1937).

The content of aldehydo or keto forms of the reducing sugars at mutarotational equilibrium has been estimated by "instantaneous" reaction with hydrogen cyanide, and polarographic, infrared, and circular dichroistic techniques. The values obtained by Cantor and Peniston (1940), using polarography, are widely cited, but it has been suggested (Capon and Overend 1960) that because the polarographic reduction is not diffusion-controlled, the results obtained by Cantor and Peniston must depend upon some process other than oxidation-reduction at the mercury surface. Nevertheless, the results roughly parallel the conformational instability of the sugars studied and suggest that the aldehydo form of glucose is present to the extent of about 0.003%. The amount of aldehydo galactose and mannose is about three times as great. Keto-D-fructose is reported to be present at equilibrium at a concentration of less than 0.4% (Swenson and Barker 1971) to about 2% (Avigad *et al.* 1970).

The distribution of fructose isomers is of special importance in food chemistry. However, reliable information on the intrinsic chemistry of this sugar is meager and contradictory. Its mutarotational behavior is difficult to assess directly because only one crystalline form is known (β-D-fructopyranose).

Upon finding that the β-D-fructopyranose isomer is not fermented by bakers' yeast, whereas the β-D-furanose form is fermented, Gottschalk (1945) was able to demonstrate that the fermentable furanose isomer is present in an equilibrium mixture at 25° at a concentration of about 22%. Using rapid invertase hydrolysis of sucrose, Andersen and Degn (1962) calculated that an equilibrium solution of fructose contains 31.56% furanose and 68.44% pyranose form. They

also calculated the specific rotation of β-D-fructofuranose to be −4.58° at 25°. These calculations are based on the premise that only two forms of fructose are present at mutarotational equilibrium. If this is not the case, then the calculations may be in error.

The indirect determination of the specific rotation of β-D-fructofuranose made by earlier students of fructose chemistry strongly suggests the need for at least one additional isomer. For example, Hudson (1909), using the technique of rapid invertase hydrolysis of sucrose, known to possess the β-D-fructofuranose moiety, calculated that the specific rotation of the furanose isomer over the temperature range 0° to 17° is about +17°, a result that can be confirmed by calculations from other data (Levi and Purves 1949). If a 2-component equilibrium is assumed, and Gottschalk's value of 22% is used for the content of the β-D-furanose isomer, the calculation

$$0.88_{Pyr}(-133°) + 0.22_{Fur}(+17°) = -113°$$

yields a value for the equilibrium specific rotation that is not in accord with the observed equilibrium specific rotation for D-fructose (−92°). These data, therefore, suggest that D-fructose has at least 3 isomers present in significant concentration at mutarotational equilibrium.

By applying the gas chromatographic separation procedure of Bentley and Botlock (1967) to the per-*O*-trimethylsilyl ethers of fructose, it is possible to distinguish 3 isomeric forms of D-fructose at mutational equilibrium (Shallenberger 1973). Results obtained at 15° are shown in Fig. 4.3. The predominant isomer, at a concentration of 76 mole percentage, is β-D-fructopyranose. The second most abundant isomer is identical with the fructose form instantly liberated from sucrose when that sugar is subjected to hydrolysis by invertase. Its concentration is 20 mole percentage. The third component, at a concentration of 4 mole percentage, has a calculated specific rotation of about +122°. Since keto-D-fructose should have very little rotatory power, and the occurrence of *cis*-hydroxymethylene substituents on the 5-membered ring of α-D-fructofuranose seems very unfavorable, the third fructose component is probably α-D-fructopyranose.

It has long been suspected that D-fructose exhibits complex mutarotation, although this is not readily deduced from optical rotatory data. A plot of change in the mole percentage of the per-*O*-trimethylsilyl derivative of β-D-fructopyranose, as monitored by gas chromatography, clearly shows, however, that the mutarotation is indeed biphasic (Fig. 4.4). Since a change of only 3 to 4 mole percentage of fructose is involved in the second phase of the mutarota-

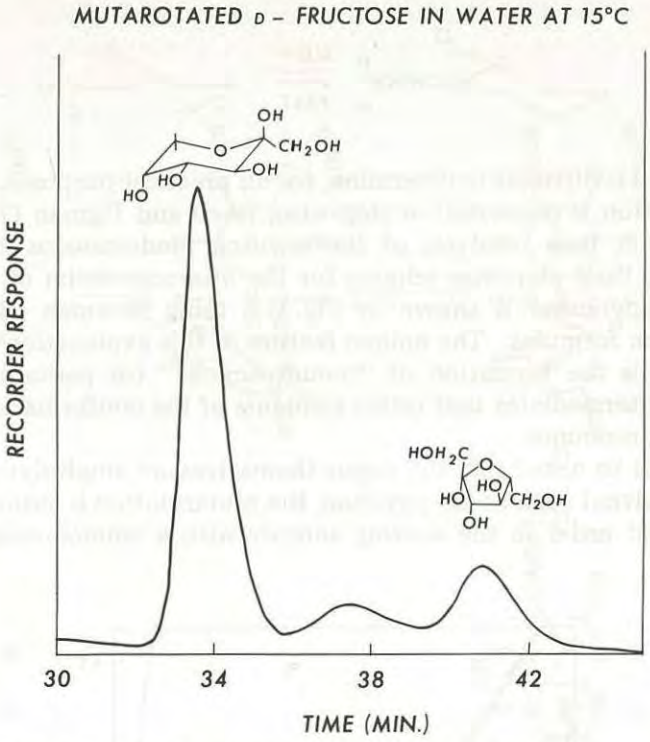
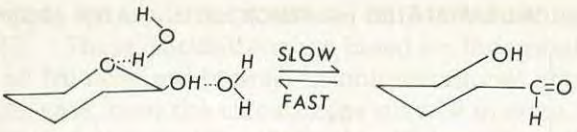


FIG. 4.3. GAS CHROMOTOGRAPHIC SEPARATION OF THE PER-O-TRIMETHYLSILYL ETHERS OF FRUCTOSE AT MUTAROTATIONAL EQUILIBRIUM

tion, the effect on optical rotatory power is obscure. If the slow phase of biphasic mutarotation generally applies to pyranose-pyranose interconversion, the plot shown in Fig. 4.4 is further evidence for a small amount of an α-D-fructopyranose isomer at equilibrium.

Mechanism of Mutarotation

During mutarotation, a proton adds to the sugar ring oxygen atom, and a proton is subtracted from the anomeric hydroxyl group. The reaction is, therefore, catalyzed by both acids and bases simultaneously (a concerted reaction), or in a step-wise manner (Isbell *et al.* 1969). The most favorable environment for the mutarotation reaction is, therefore, an amphoteric system. Water is, of course, an ampholyte, and may catalyze the reaction by either the concerted or the step-wise mechanism. The concerted mechanism uses two molecules of water, one acting as an acid and the other as a base:



While it is difficult to determine, for all practical purposes, whether the reaction is concerted or step-wise, Isbell and Pigman (1969) deduced that base catalysis of the reaction predominates in neutral solution; their step-wise scheme for the interconversion of α-D- and β-D-glucopyranose is shown in Fig. 4.5 using Newman (Böeseken) projection formulas. The unique feature of this explanation of mutarotation is the formation of "pseudo-acyclic" (or perhaps pseudo-cyclic) intermediates that retain elements of the conformation of the parent compounds.

It must be noted that the sugars themselves are ampholytes, and in a basic solvent such as dry pyridine, the mutarotation is autocatalytic, being first order in the starting anomer with a unimolecular depen-

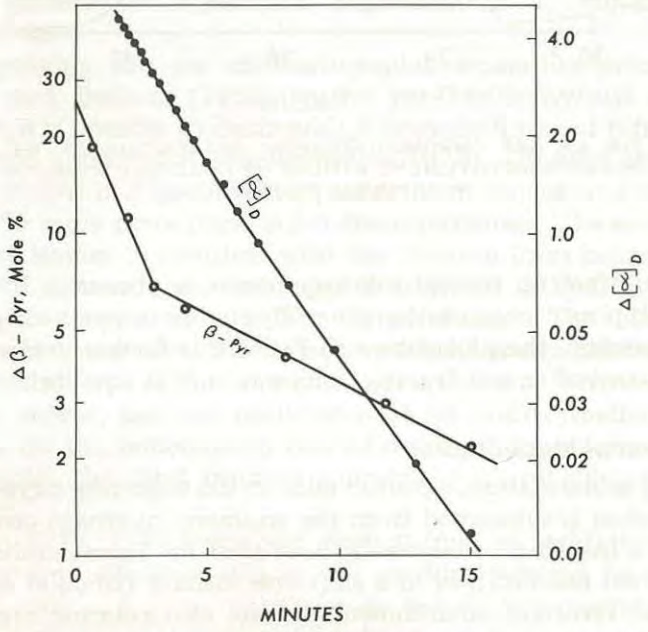
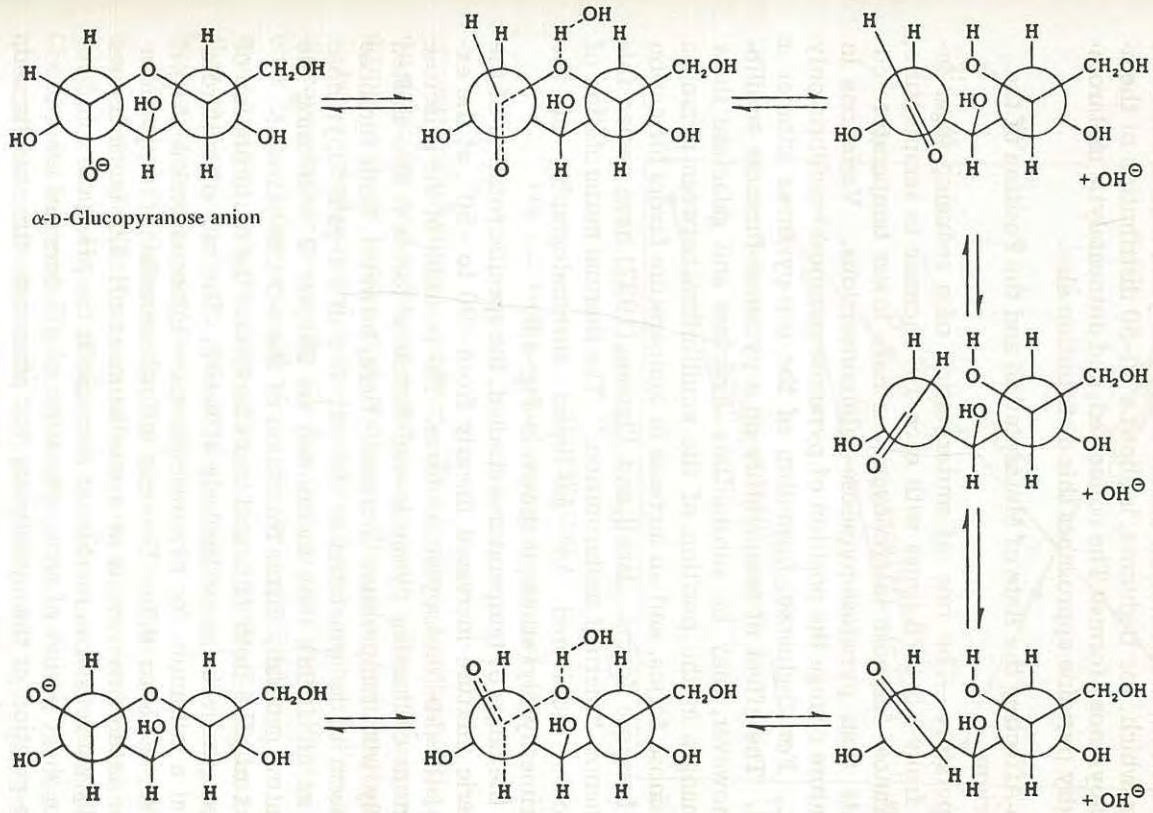


FIG. 4.4. MUTAROTATION OF β-D-FRUCTOPYRANOSE AS MONITORED BY OPTICAL ROTATION AND CHANGE IN THE MOLE PERCENT OF STARTING TAUTOMER AS MONITORED BY SEPARATION OF PER-O-TRIMETHYLSILYL ETHERS SEPARATED BY GAS CHROMATOGRAPHY



Isbell et al. (1969)

FIG. 4.5. INTERCONVERSION OF α- AND β-D-GLUCOPYRANOSE IN ALKALINE SOLUTION

Reproduced with permission of Elsevier Publishing Company.

dence on the total concentration of sugar (Hill and Shallenberger 1969).

As a consequence of their amphoteric nature, when a reducing sugar is melted, mutarotation occurs rapidly to yield a distribution of anomers which, for D-glucose, is about a 50–50 distribution of the α- and β-D-pyranose forms. The concerted, and autocatalytic mutarotation in dry pyridine approaches this distribution also.

Factors Affecting the Rate of Mutarotation and the Position of the Equilibrium

Temperature.—The rate of mutarotation of a reducing sugar increases from 1.5 to 3 times with each 10° increase in temperature, and pyranose-furanose interconversions have lower temperature coefficients than pyranose-pyranose interconversions. Variations in temperature change the position of pyranose-pyranose equilibria only slightly. For D-glucose, formation of the α-D-pyranose anomer is favored. The effect of temperature on a pyranose-furanose equilibrium, however, may be substantial. Fructose and galactose show large changes in the position of the equilibrium between pyranose and furanose forms, and an increase in temperature favors formation of the furanose forms. Isbell and Pigman (1937) have named this phenomenon "thermal mutarotation." The thermal mutarotation of D-fructose, monitored by gas-liquid chromatography of the per-*O*-trimethylsilyl ethers, is shown in Fig. 4.6.

Over the range of temperatures studied, the specific rotation of the tautomeric mixture increased linearly from −90 to −50°, at the expense of the β-D-fructopyranose form. The position of the galactose equilibrium containing pyranose and furanose forms is also shifted markedly with temperature increase. Here, however, both furanose forms seem to be generated at the expense of β-D-galactopyranose (Acree *et al.* 1968). As mentioned in Chapter 3, increasing the temperature generally favors formation of the α-D-aldohexoses.

Effect of pH.—Both acids and bases accelerate the mutarotation of the sugars, bases being particularly effective. The rate of mutarotation is at a minimum for pyranose-pyranose interconversion at a pH range from 2.5 to 6.5. The rate of mutarotation for pyranose-furanose interconversion is at a minimum at pH 4.0, but increases markedly with either an increase or decrease in the pH of the solution. Within a short period of time, alteration of pH does not seem to affect the position of the equilibrium, but after some time has elapsed, secondary reactions take on added importance.

Effect of Enzymes.—In dynamic biological systems, a certain amount of glucose is necessary for metabolic functions. An enzyme known as *mutarotase* is present which accelerates the inter-

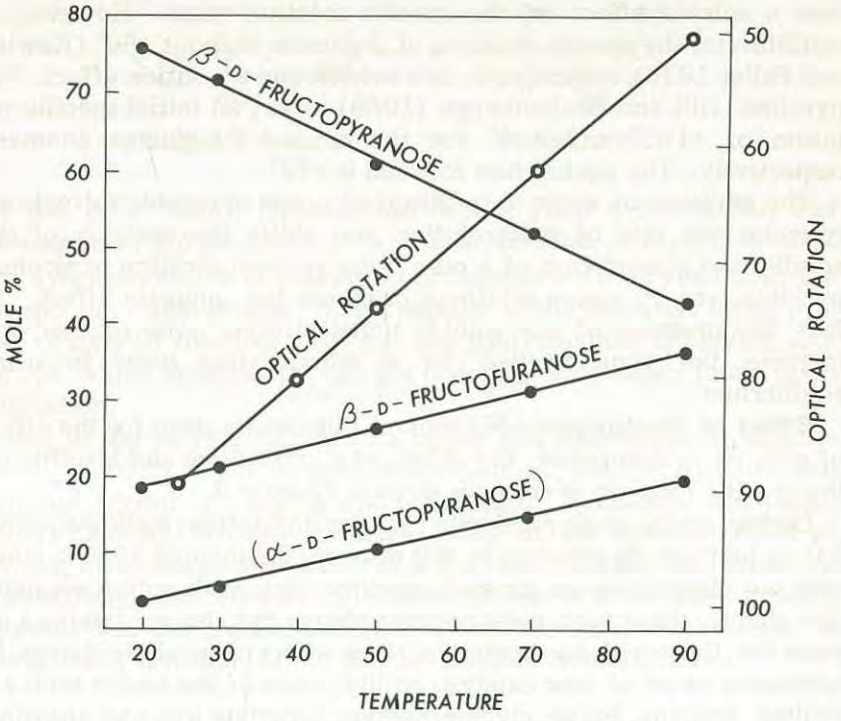


FIG. 4.6. THERMAL MUTAROTATION OF D-FRUCTOSE

conversion of glucose anomers. The enzyme also has some activity on galactose and reducing disaccharides with a free glucose anomeric group, such as lactose and maltose. The role of mutarotase seems to be to speed conversion to the metabolizable anomer. For example, glucose oxidase is specific for β-D-glucose, but the oxidation of glucose is much more rapid than can be explained by the intrinsic mutarotation rate (Bentley and Neuberger 1949). It is also believed that the enzyme has a role in the active transport of sugars across biological membranes (Keston 1964).

Effect of the Solvent.—Both the rate of mutarotation and the position of the equilibrium are affected by the polarity of the solvent, which of course also affects the specific rotation, as discussed in Chapter 3. In general, as the polarity of the solvent is decreased, the mutarotation rate decreases, and the position of the equilibrium for D-glucose shifts toward the 50–50 distribution of anomers found in glucose melts. In absolute ethanol and methanol, the initial specific rotations of α-D- and β-D-glucose are +115° and +17°, respectively. These values are close to those found in water solution, but do indi-

cate a solvent effect on the specific rotation value. However, at equilibrium, the specific rotation of D-glucose is about +66° (Rowley and Bailey 1940), indicating both a solvent and a position effect. For pyridine, Hill and Shallenberger (1969) report an initial specific rotation of +152° and +11° for the α- and β-D-glucose anomers, respectively. The equilibrium rotation is +72°.

The addition of water to solutions of sugars in organic solvents accelerates the rate of mutarotation and shifts the position of the equilibrium toward that of a pure water system; addition of alcohol, pyridine, etc. to water solutions produces the opposite effect. In fact, the presence of any soluble nonelectrolyte, even sucrose, will increase the time needed for a mutarotating sugar to reach equilibrium.

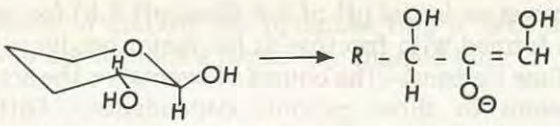
Effect of Electrolytes.—No general rule can be given for the effect of salts on mutarotation, the effect of alkali halides and bisulfite on the specific rotation of sugars is given in Chapter 3.

Certain amino acids accelerate sugar mutarotation; histidine, at pH 6.0, is particularly effective in this respect (Westheimer 1937). However, no distinction can be made among amino acids with a net negative charge, those with a net negative charge but also containing a dipolar ion, those with no charge, and those with a net positive charge. In increasing order of base catalysis ability, some of the amino acids are proline, arginine, lysine, glycine, alanine, aspartate ion, and histidine.

ENOLIZATION AND ISOMERIZATION REACTIONS

Some time after the mutarotation of the aldose and ketose sugars has taken place, a second intrinsic reaction of the sugars, also catalyzed by both acids and bases, takes place. It is known as the Lobry de Bruyn-Alberda van Ekenstein transformation, and involves both the enolization of an aldehydo or keto sugar and the subsequent formation of functional group isomers and disastereoisomers. As an example, glucose, fructose, and mannose establish an equilibrium, through a hypothetical 1,2-enediol intermediate (Fig. 4.1). However, a single acyclic intermediate enolic form cannot account for all the known details of the behavior of sugars in solution, although a "single" intermediate form is shown in Fig. 4.1. Isbell *et al.* (1969) suggest that after mutarotation, a α-D-glucopyranose yields a *cis*-enediol, and β-D-glucopyranose yields a *trans*-enediol:

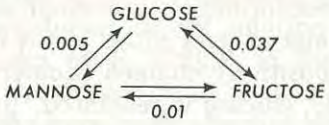
α-D-GLUCOPYRANOSE *CIS*-ENEDIOL



β-D-GLUCOPYRANOSE *TRANS*-ENEDIOL

On the other hand, β-D-mannose would yield a *cis*-enediol, and α-D-mannose, a *trans*-enediol. A ketose, because of the presence of two hydrogen atoms at carbon atom number 1 would yield both the *cis*- and the *trans*-enediol. Thus, because of the anomeric forms possible, sugars in solution yield *cis*- and *trans*-enediols in varying proportion, which explains, in part, the distribution of sugars found upon enolization.

Due to the varying proportions of *cis*- and *trans*-enediols, and the varying stability of the favored conformations, glucose, fructose, and mannose appear in varying concentrations. MacLaurin and Green (1968) showed, through a kinetic study of the transformation of glucose, mannose, and fructose, that the rate constants for the reversible pathways are identical, and that evaluation of these rate constants suggests that the glucose-mannose epimerization reaction is not as energetically favorable as the glucose-fructose isomerization:



The relative rates of enolization of glucose, mannose, and fructose are 1.0, 0.5, and 10.7, respectively, and are of the same order as the relative rates of disappearance of these compounds before reverse and side reactions become important. In line with these results, Topper and Stetten (1951) found that the mannose generated by the action of alkali on glucose at 25° to 30° is derived exclusively from fructose. It follows, therefore, that after a period of time in dilute alkaline solution, any one sugar will yield a mixture of sugars. Sowden and Schaffer (1952A) found glucose 66.5%, fructose 28.6%, and mannose 0.8% after 21 days at 25° in lime water. Starting with other sugars, such as D-galactose, the epimer D-talose and the ketose D-tagatose are formed.

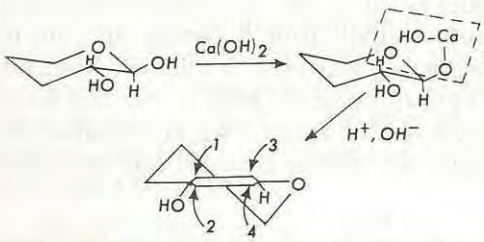
Factors Affecting Enolization and Isomerization Reactions

Effect of pH.—The transformations just described are usually associated with an alkaline pH, but they also occur, with less facility, in acid solutions. Englis and Hanahan (1945) found that after auto-

claving glucose at an initial pH of 6.6 (final pH 5.5) for only 1 hr, 7% ketoses were formed with fructose as the major product.

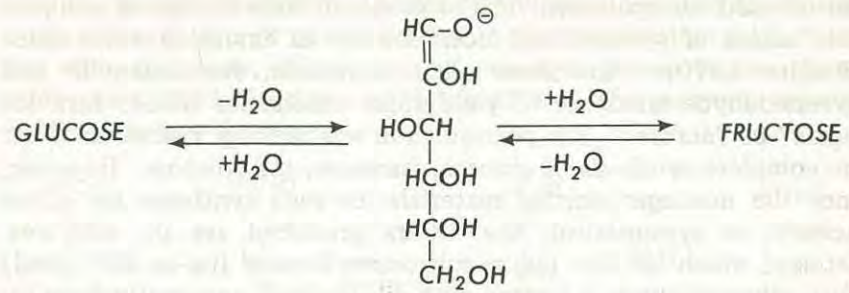
Effect of Base Cations.—The course of events for the action of alkali on sugars seems to show cationic dependence. Different intermediates are found when monovalent or divalent cations of bases are employed. Kusin (1936) found that the *initial* course of the reaction is different with monovalent and divalent bases at 0.5*N* concentration at low temperatures (35 to 37°). Apparently no difference is found at higher temperatures. At low temperature $Ca(OH)_2$ and NaOH readily cause enolization, but the enolic compounds formed are not the same. To account for the fact that glucose in $Ca(OH)_2$ solution yielded only mannose after 24 hr, whereas glucose in NaOH solution afforded only fructose, Kusin (1936) postulated the formation of a cyclic, comparatively stable enol without opening the pyranose ring.

Kusin (1936) visualized the formation of an initial calcium saccharate by removal of a proton from the anomeric OH group, and the formation of water. In an excess of $Ca(OH)_2$, the saccharate would dissociate only slightly. $Ca(OH)_2$ would then split from the saccharate, removing the anomeric and the neighboring methine proton, to yield the cyclic enol. Since a double bond is created in the ring, the ring oxygen atom and carbon atoms 1, 2, and 3 lie in the same plane, and substituents at each end of the double bond also lie in this plane. The conformation generated would be that of a distorted chair. When water is again added to the molecule, with the OH group attacking the positively charged anomeric carbon atoms from "above" the molecule, glucose is generated. If the approach is from "below" the molecule, mannose is generated. Should the addition of water in a concerted manner occur from either direction, the β-D-glucose and the α-D-mannose formed would (interestingly) immediately yield the favored anomer and the favored chair conformation for these sugars:



ADDITION OF A PROTON AT 1 YIELDS GLUCOSE.
ADDITION OF A PROTON AT 2 YIELDS MANNOSE.
ADDITION OF AN OH GROUP AT 3 YIELDS A β-ANOMER.
ADDITION OF AN OH GROUP AT 4 YIELDS AN α-ANOMER.

In the presence of NaOH, the pyranose ring is broken to form an acyclic enol in the form of a negatively charged ion:



Since the terminal carbon atom has a negative charge, it will attract the proton when water is added to the molecule, while the OH group will migrate to the adjacent carbon atom, resulting in the formation of fructose. It must be emphasized that these results were obtained at 0.5*N* alkali concentrations. At an alkali concentration of 0.035*N*, Sowden and Schaffer (1952A) could detect no difference in the catalysis of the isomerization of glucose using calcium hydroxide and sodium hydroxide. The studies of MacLaurin and Green (1969), which showed an energetic barrier between glucose and mannose epimerization, were conducted in 1*M* solution of NaOH. It is also significant that Kainuma *et al.* (1968), in the development of an industrial process for the formation of fructose from glucose, found that Li, Na, and K ions were more effective than the alkaline-earth metals. Also, at the same pH and metal-ion concentrations, these authors could find no difference among the actions of the ions.

Other evidence for a cation effect on the course of the enolization reaction has been discerned for lead versus potassium hydroxide, as monitored by optical rotation (Speck 1958), and the rate of disappearance of mannose in calcium hydroxide solution is much greater than in solutions of barium hydroxide or sodium hydroxide (Sowden and Schaffer 1952B).

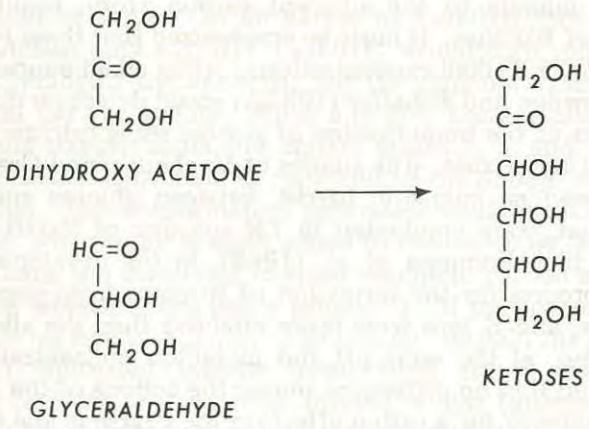
The effect of the ammonium ion (NH_4^+) presents a very special case, not only for enolization and isomerization phenomena, but also for degradation, fragmentation, and polymerization reactions. It will be taken up in detail in Chapter 6.

Applications of Isomerization Reactions

The sugar transformation reactions have several applications to foods. One of the most obvious is the generation of fructose, which has higher sweetening power, from the readily available glucose of

commerce, "dextrose." This can be brought about either enzymically, or by the use of alkalies.

Since the enolization condensation reactions are reversible, they can be used for synthesis. The subject is of current interest as a possible means of synthesizing foods for use in exploring outer space (Shapira 1970). In dilute alkali solutions, formaldehyde and glyceraldehyde condense to yield sugar substances called "formose sugars" or "acrose." The phenomenon was used by Fischer to effect the complete synthesis of glucose, mannose, and fructose. However, since the nonsugar starting materials for such syntheses are either racemic or symmetrical, the sugars produced are DL mixtures. Ketoses, which are the major substances formed (up to 92% yield) when glyceraldehyde is treated with dilute alkali, apparently form by the condensation of glyceraldehyde with generated dihydroxyacetone (Berl and Feazel 1951):



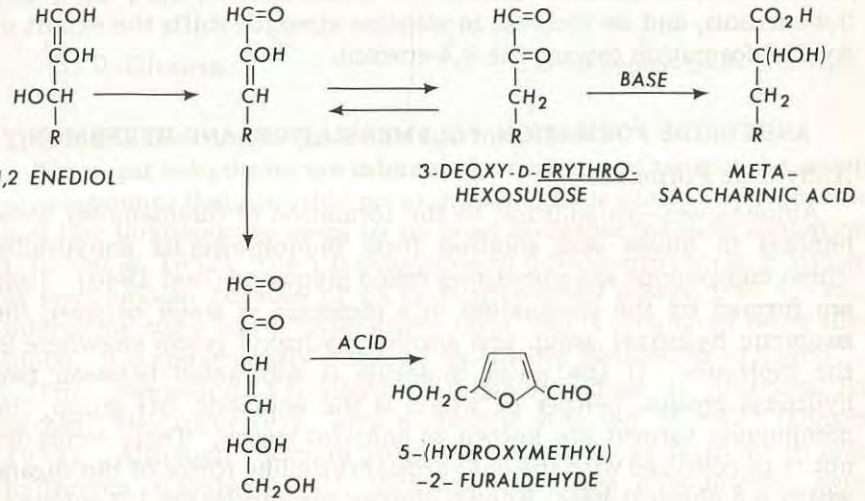
The dihydroxyacetone arises from an equilibrium with glyceraldehyde in alkaline solution. Since symmetrical reagents are used at the outset, it is possible for four ketohexoses to be formed, but the two major components of the reaction are DL-fructose and DL-sorbose. For production of a satisfactory food resolution of the racemic mixture would be required, since the L-sugars are generally inert in biochemical reactions, or are even potential antimetabolites.

DEGRADATION REACTIONS

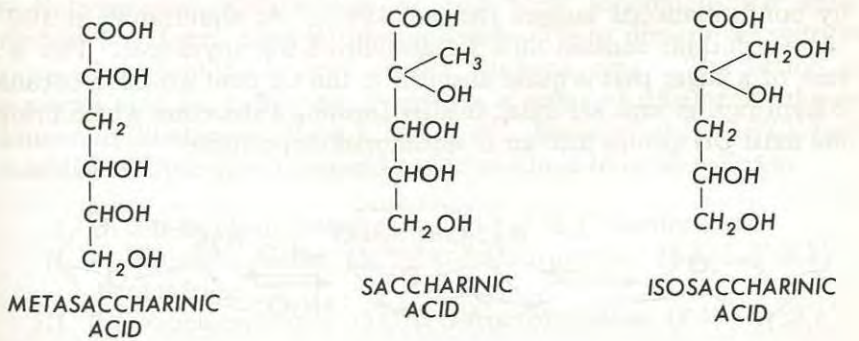
Principal Action of Acids and Bases

In either acid or base, enolization of the acyclic form of a reducing sugar may progress "down" the carbon chain. The 2,3-enediol is a form of 3-deoxy-D-*erythro*-hexosulose (3-deoxyglucosone). In acid

solution, the formation of the enolic form is envisaged as a dehydration reaction, and in basic solution as a β-elimination reaction. Further dehydration in acid media leads to the formation of furfural compounds. In alkaline solution, the deoxyaldulose is believed to undergo a benzilic acid type of rearrangement to yield saccharinic acid. Starting with D-glucose, this course of events is shown below (Isbell 1944; Anet 1964; Feather and Harris 1970). The products are also obtained from D-fructose.



The principal products obtained by the action of bases on the hexoses are a series of 6-carbon deoxy acids and lactic acid (Sowden 1957). The 6-carbon acids are found in 3 isomeric forms, depending on the concentration of alkali. These are:



There are 8 isomers possible for the metasaccharinic acids (3-deoxyhexonic acids) and the saccharinic acids (2-C-methyl pentonic acids);

4 isomers are possible for the isosaccharinic acids (3-deoxy-C-hydroxymethyl pentonic acids).

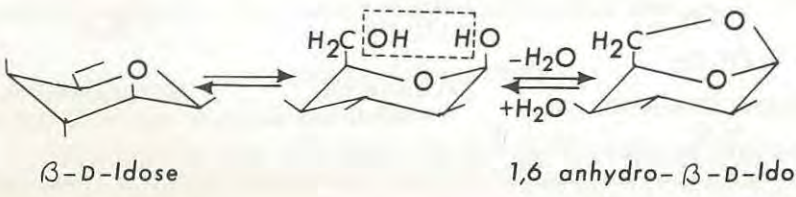
One rule is that for pentose or higher sugars, the configuration after carbon atom number 3 is not disturbed in the formation of saccharinic acids. In dilute alkaline solution, saccharinic acid formation is favored, whereas in concentrated alkali, iso- and metasaccharinic acid formation is favored. Overall, the enolization rate of a sugar is proportionate to the concentration of the hydroxyl ion; an increase in alkali concentration favors the formation of 1,2-, 2,3-, and 3,4-enediols, and an increase in alkaline strength shifts the extent of enediol formation toward the 3,4-enediol.

ANHYDRIDE FORMATION, POLYMERIZATION, AND REVERSION

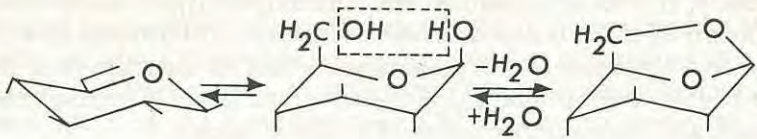
Anhydride Formation

Aldohexoses.—In addition to the formation of furaldehydes, aldohexoses in dilute acid solution form monomolecular anhydrides. These compounds are sometimes called glycosans (Peat 1946). They are formed by the elimination of a molecule of water between the anomeric hydroxyl group and another hydroxyl group elsewhere in the molecule. If the water molecule is subtracted between two hydroxyl groups, neither of which is the anomeric OH group, the compounds formed are known as *anhydro* sugars. These terms are not to be confused with the *anhydrous* crystalline forms of the sugars, which is a physical state. Known glucose anhydrides are 1,2-anhydro-α-D-glucopyranose, 1,6-anhydro-β-D-glucofuranose, and 1,6-anhydro-β-D-glucopyranose (levoglucosan).

The most commonly encountered sugar anhydrides are those of the 1,6-anhydro-β-D-aldohexopyranoses. The extent to which the anhydrides form is a clear-cut example of an equilibrium controlled by conformational factors (Angyal 1965). At equilibrium at 100°, idose solutions contain 80% 1,6-anhydro-β-D-idopyranose. This is a case of a sugar that is quite unstable in the C1 conformation because 3 hydroxyl groups are axial, readily forming a structure which brings the axial OH groups into an all-equatorial disposition:



On the other hand, β-D-glucopyranose, with all OH groups in the favored all-equatorial position, forms the 1,6 anhydride with the greatest difficulty:



β-D-Glucose 1,6 anhydro-β-D-Glucose

This is also the case for galactose and mannose.

The sugar anhydrides are internal glycosides, and represent a group of compounds that probably occur in foods, since dilute acid solutions and low temperatures seem to be most favorable for their formation (Zissis *et al.* 1957). The 1,6 anhydride of β-D-glucopyranose is known as levoglucosan because of its optical rotatory power (−67°). This interesting sugar has a bitter-sweet taste, and is capable of being sublimed. It is conveniently prepared by the dry distillation of starch.

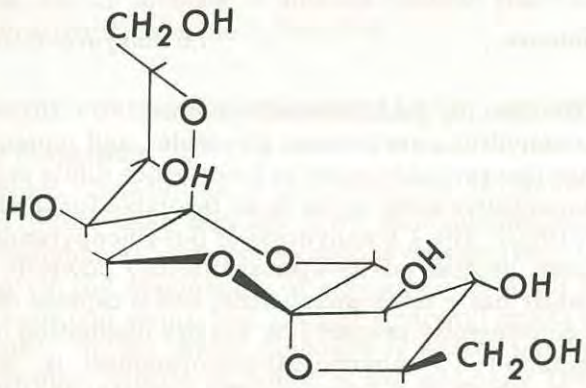
The compound 1,6-anhydro-β-D-glucofuranose is, interestingly enough, dextrorotatory, with a specific rotation of +42° (Dimler 1952). Although furanoid anhydrides seem to be resistant to acid hydrolysis (Dimler 1952), they are not often encountered, even though they have the same ring system as the pyranoid anhydrides (a 5-membered ring fused to a 6-membered ring). Angyal and Dawes (1968) suggest that this is because a furanoid ring has 4 carbon atoms carrying substituents other than H-atoms, which lends an element of instability to the compounds.

Ketohexoses.—Ketohexoses do not form monomolecular anhydrides, but form dianhydrides instead. These dimeric anhydrides possess an acid-stable and central dioxane ring. The action of aqueous acid on D-fructose generates a series of diheterolevulosans known as diheterolevulosan I, II, III, etc. Binkeley *et al.* (1973) have established their structures and conformations to be as follows:

I. Di-α-D-fructopyranose (1C, C1) 1,2′:2,1′ dianhydride.
II. α-D-Fructopyranose (1C)-β-D-fructofuranose (E3)-1,2′:2,1′ dianhydride.
III. β-D-Fructopyranose (1C)-β-D-fructofuranose (E4)-1,2′:2,1′ dianhydride.
IV. Di-β-D-fructopyranose (1C, 1C)-1,2′:2,1′ dianhydride.

The dianhydride central dioxane rings were found to be in conformations which minimized interactions between the two fructose structures.

A related series of fructose dianhydrides, known as the di-D-fructose anhydrides I, II, etc. are obtained from acid-hydrolyzed inulin. The better known of these is di-D-fructose anhydride I (McDonald 1946). Lemieux and Nagarajan (1964) established that its structure is 1′,2-anhydro-[1-(α-D-fructofuranosyl)-β-D-fructofuranose]. The ring conformations, and the structure assigned is shown below:



DI-D-FRUCTOSE ANHYDRIDE I

Reversion

Upon standing in dilute acid solution, at a high concentration of sugar, and at ambient temperature, a series of disaccharides are formed. The phenomenon is known as "reversion," since the type of product was first encountered during the acid hydrolysis of starch. Among the disaccharides formed, α-D-1,6 linkages predominate, followed by β-D-1,6 linkages. For D-glucose in 0.082*N* hydrochloric acid, Thompson *et al.* (1954) found the following disaccharides.

β,β-Trehalose (β-D-Glucopyranosyl β-D-glucopyranoside),	0.1%
β-Sophorose (2-*O*-β-D-Glucopyranosyl-β-D-glucopyranose),	0.2%
β-Maltose (4-*O*-α-D-Glucopyranosyl-β-D-glycopyranose),	0.4%
α-Cellobiose (4-*O*-β-D-Glucopyranosyl-α-D-glucopyranose),	0.1%
β-Cellobiose (4-*O*-β-D-Glucopyranosyl-β-D-glucopyranose),	0.3%
β-Isomaltose (6-*O*-α-D-Glucopyranosyl-β-D-glucopyranose),	4.2%
α-Gentiobiose (6-*O*-β-D-Glucopyranosyl-α-D-glucopyranose),	0.1%
β-Gentiobiose (6-*O*-β-D-Glucopyranosyl-β-D-glucopyranose),	3.4%

In addition to these compounds, Peat (1958) found laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucose), nigerose (3-*O*-α-D-

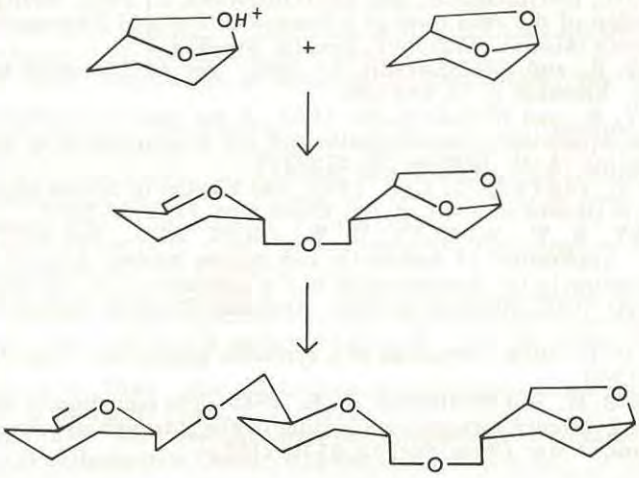
glucopyranosyl-D-glucose) and kojibiose (2-*O*-α-D-glucopyranosyl-D-glucose). In beer (Aso *et al.* 1961), the origin of sugars such as kojibiose and nigerose, to the extent of 3.3% of the total sugar, is clearly due to reversion.

Bishop (1956) pointed out that the linkages formed in the reversion of hexoses follow an order that is either determined by the reactivity of the OH groups, or is directed by differences in the acid stability of the linkages, once formed. In either case, the approximate reactivities of the hexopyranose OH groups, as measured by the ability to form disaccharides, is $1 > 6 > 4 > 3 > 2$. The relative acid stabilities of the glycosidic linkages are $(1,6) > (1,4) > (1,3) > (1,2)$. In line with these results, Wolfrom *et al.* (1951) found that the α-1,4-glucose linkage is hydrolyzed by acid 4 times as rapidly as the α-1,6 linkage.

Polymerization

While the products of the reversion reaction of the acid hydrolysis of polysaccharides are mainly disaccharides, the formation of "polyoses" with a fairly high degree of polymerization is possible at low moisture levels (Pascu and Mora 1950). The polymeric linkages formed in the reversion of hexoses by acid are primarily α-1,6 and α-1,4 (Bishop 1956), and the polymers generated are therefore highly branched.

The relative lack of 1,1 linkages is intriguing since these are the most "reactive" OH groups. DaSilva Carvalho *et al.* (1959) propose that levoglucosan is formed first and that polymerization proceeds in part through some intermediate related structurally to 1,2-anhydroglucopyranose:



These "glucans" receive occasional interest for a variety of reasons, one of which is their use as possible blood plasma extenders, when the molecular weight attains a value of 75,000 or higher. They have also been shown to have serological activity against pneumococcus polysaccharides and are remarkably resistant to enzyme hydrolysis (Kent 1953). Upon acid hydrolysis, those polymers formed under relatively mild conditions yield glucose as the sole product providing, of course, that glucose was used as the starting material.

BIBLIOGRAPHY

ACREE, T. E., SHALLENBERGER, R. S., and MATTICK, L. R. 1968. Mutarotation of D-galactose. Tautomeric composition of an equilibrium solution in pyridine. Carbohyd. Res. *6*, 498–502.

ACREE, T. E., SHALLENBERGER, R. S., LEE, C. Y., and EINSET, J. W. 1969. Thermodynamics and kinetics of D-galactose tautomerism during mutarotation. Carbohyd. Res. *10*, 355–360.

ANDERSON, B., and DEGN, H. 1962. Determination of the optical rotation of β-fructofuranose by means of kinetic measurements. Acta Chem. Scand. *16*, 215–220.

ANET, E. F. L. J. 1964. 3-Deoxyglycosuloses (3-deoxyglycosones) and the degradation of carbohydrates. Advances in Carbohydrate Chem. *19*, 181–218.

ANGYAL, S. J. 1965. Conformational analysis in carbohydrate chemistry. *In* Conformational Analysis, E. Eliel *et al.* (Editors). Interscience Publishers, New York.

ANGYAL, S. J. 1968. Conformational analysis in carbohydrate chemistry. I. Conformational free energies. The conformations and α:β ratios of aldopyranoses in aqueous solution. Aust. J. Chem. *21*, 2737–2746.

ANGYAL, S. J., and DAWES, K. 1968. Conformational analysis in carbohydrate chemistry. II. Equilibria between reducing sugars and their glycosidic anhydrides. Aust. J. Chem. *21*, 2747–2760.

ASO, K., WATANABE, T., SASAKI, K., and MOTOMURA, Y. 1961. Studies on beer. I. Sugar composition of beers. Tohoku J. Agr. Res. *12*, 261–268.

AVIGAD, G., ENGLARD, S., and LISTOKOWSKI, I. 1970. Evaluation of the proportion of the keto form of D-fructose and related 2-ketohexoses present in aqueous solution. Carbohyd. Res. *14*, 365–373.

BENTLEY, R., and NEUBERGER, A. 1949. The mechanism of the action of notatin. Biochem. J. *45*, 584–590.

BENTLEY, R., and BOTLOCK, N. 1967. A gas chromatographic method for analysis of anomeric carbohydrates and for determination of mutarotation coefficients. Anal. Biochem. *20*, 312–320.

BERL, W. G., and FEAZEL, C. E. 1951. The kinetics of hexose formation from trioses in alkaline solution. J. Am. Chem. Soc. *73*, 2054–2057.

BINKELEY, R. W., BINKLEY, W. W., GREY, A. A., and WICKBERG, B. 1973. Application of carbon-13 and proton nuclear magnetic resonance spectroscopy in the determination of the structure of the diheterolevulosans. Abstracts 164th National Meeting, American Chemical Society, Abs. Carb. No. 15.

BISHOP, C. T. 1956. Structure of a synthetic polyxylose. Can. J. Chem. *34*, 1255–1260.

CANTOR, S. M., and PENISTON, Q. P. 1940. The reduction of aldoses at the dropping mercury cathode: Estimation of the aldehydo structure in aqueous solutions. J. Am. Chem. Soc. *62*, 2113–2121.

CAPON, B., and OVEREND, W. G. 1960. Constitution and physiochemical properties of carbohydrates. Advances in Carbohydrate Chem. *15*, 11–51.

DESILVA CARVALHO, J., PRINS, W., and SCHUERCH, C. 1959. Addition polymerization of anhydrosugar derivatives. I. A polyanhydroglucose. J. Am. Chem. Soc. *81*, 4054–4058.

DIMLER, R. J. 1952. 1,6-Anhydrohexofuranoses, a new class of hexosans. Advances in Carbohydrate Chem. *7*, 37–52.

ENGLIS, D. T., and HANAHAN, D. J. 1945. Changes in autoclaved glucose. J. Am. Chem. Soc. *67*, 51–54.

FEATHER, M., and HARRIS, J. F. 1970. On the mechanism of conversion of hexoses into 5-(hydroxymethyl)-2-furaldehyde and metasaccharinic acid. Carbohyd. Res. *15*, 304–309.

GOTTSCHALK, A. 1945. Yeast hexokinase and its substrates D-fructofuranose and D-glucose. Nature *156*, 540–541.

HILL, A. S., and SHALLENBERGER, R. S. 1969. Autocatylic mutarotation of D-glucose in pyridine. Carbohyd. Res. *11*, 541–545.

HUDSON, C. S. 1909. The inversion of cane sugar by invertase. III. J. Am. Chem. Soc. *31*, 655–664.

ISBELL, H. S. 1944. Interpretation of some reactions in the carbohydrate field in terms of consecutive electron displacement. J. Res. Nat. Bur. Stds. *32*, 45–59.

ISBELL, H. S., and PIGMAN, W. W. 1937. Bromine oxidation and mutarotation measurements of the alpha- and beta- aldoses. J. Res. Nat. Bur. Stds. *18*, 141–194.

ISBELL, H. S., and PIGMAN, W. W. 1938. Pyranose-furanose interconversions with reference to the mutarotations of galactose, levulose, lactulose, and turanose. J. Res. Nat. Bur. Stds. *20*, 773–798.

ISBELL, H. S., and PIGMAN, W. W. 1969. Mutarotation of sugars in solution. Part II. Catalytic processes, isotope effects, reaction mechanisms and biochemical aspects. Advances in Carbohydrate Chem. and Biochem. *24*, 13–65.

ISBELL, H. S., FRUSH, H. L., WADE, C. W. R., and HUNTER, C. E. 1969. Transformations of sugars in alkaline solutions. Carbohyd. Res. *9*, 163–175.

KAINUMA, K., TADOKORO, K., and SUZUKI, S. 1968. Isomerization of dextrose into fructose. III. Effects of cations on the isomerization of dextrose. Nippon Nogei Kagaku Kaishi *42*, 173–177.

KENT, P. W. 1953. Chemical synthesis of hexose polymers. Biochem. J. *55*, 361–364.

KESTON, A. S. 1964. Kinetics and distribution of mutarotases and their relation to sugar transport. J. Biol. Chem. *239*, 3241–3251.

KUSIN, A. 1936. Enolization of sugars by different bases. Ber. *69*, 1041–1049.

LEMIEUX, R. U., and NAGARAJAN, R. 1964. The configuration and conformation of di-D-fructose anhydride. I. Can. J. Chem. *42*, 1270–1278.

LEMIEUX, R. U., and PAVIA, A. A. 1969. Substitutional and solvation effects on conformational equilibria. Effects on the interaction between opposing axial oxygen atoms. Can. J. Chem. *47*, 4441–4446.

LEVI, I., and PURVES, C. B. 1949. The structure and configuration of sucrose. Advances in Carbohydrate Chem. *4*, 1–35.

MACLAURIN, D. J., and GREEN, J. W. 1969. Carbohydrates in alkaline systems. I. Kinetics of the transformation and degradation of D-glucose, D-fructose, and D-mannose in 1*M* sodium hydroxide. Can. J. Chem. *47*, 3947–3955.

MCDONALD, E. J. 1946. The polyfructosans and difructose anhydrides. Advances in Carbohydrate Chem. *2*, 253–277.

MILLS, J. A. 1955. The stereochemistry of cyclic derivatives of carbohydrates. Advances in Carbohydrate Chem. *10*, 1–53.

MURCHAUSER, H. 1923. The influence of some normal salts on the mutarotation and specific rotation of glucose. Biochem. Z. *136*, 66-76.

PASCUE, E., and MORA, P. T. 1950. Polycondensation of D-glucose and other simple sugars in the presence of acids. J. Am. Chem. Soc. *72*, 1045.

PEAT, S. 1946. The chemistry of anhydro sugars. Advances in Carbohydrate Chem. *2*, 37-77.

ROWLEY, H. H., and BAILEY, S. D. 1940. The mutarotation of D-glucose in absolute methanol. J. Am. Chem. Soc. *62*, 2562-2563.

SHALLENBERGER, R. S. 1973. Sugar structure and taste. *In* Chemistry of Carbohydrates in Solution, R. F. Gould (Editor). Advances in Chemistry Series *117*, 256-263.

SHAPIRA, J. 1970. Physicochemical methods for the synthesis of potential foods. J. Agr. Food Chem. *18*, 992-996.

SOWDEN, J. C. 1957. The saccharinic acids. Advances in Carbohydrate Chem. *12*, 35-79.

SOWDEN, J. C., and SCHAFFER, R. 1952A. The isomerization of D-glucose by alkali in D_2O at 25°C. J. Am. Chem. Soc. *74*, 505-507.

SOWDEN, J. C., and SCHAFFER, R. 1952B. The reaction of D-glucose, D-mannose, and D-fructose in 0.035*N* sodium hydroxide at 35°C. J. Am. Chem. Soc. *74*, 499-504.

SPECK, JR., J. C. 1958. The Lobry De Bruyn-Alberda Van Ekenstein transformation. Advances in Carbohydrate Chem. *13*, 63-103.

SWEELEY, C. C., BENTLEY, R., MAKITA, M., and WELLS, W. W. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. *85*, 2497-2507.

SWENSON, C. A., and BARKER, R. 1971. Proportion of keto and aldehydo forms in solutions of sugars and sugar phosphates. Biochem. *10*, 3151-3154.

THOMPSON, A., ANNO, K., WOLFROM, M. L., and INATOME, M. 1954. Acid reversion products form D-glucose. J. Am. Chem. Soc. *76*, 1309-1311.

TIPSON, R. S., and ISBELL, H. S. 1962. Infrared absorption spectra in the study of mutarotational equilibria of monosaccharides. J. Res. Nat. Bur. Stds. *66A*, 31-58.

TOMODA, Y., and TAGUCHI, T. 1930. Effect of sodium bisulfite upon polarizing power of sugars and the use of sodium bisulfite in sugar analysis. J. Soc. Chem. Ind. (Japan) *33*, 431-441.

TOPPER, Y. J., and STETTEN, JR., D. 1951. The alkali-catalyzed conversion of glucose into fructose and mannose. J. Biol. Chem. *189*, 191-202.

WESTHEIMER, F. H. 1937. Amino acid catalysis of the mutarotation of glucose. J. Org. Chem. *2*, 431-441.

ZISSIS, E., STEWART, L. C., and RICHTMYER, N. K. 1957. The formation of 2,7-anhydro-β-D-manno-heptulopyranose. J. Am. Chem. Soc. *79*, 2593-2597.

Nutritional and Metabolic Aspects of Sugars

The physical and chemical properties of the sugars enumerated in Chapter 3 form the basis of their biochemical and nutritional properties. All sugars absorbed into the blood stream are usually converted into glucose, which is then fed into the body tissues to provide energy. Excess glucose is converted either to glycogen and kept as an energy store until needed, or to reserve fatty tissue. In periods of starvation reserve energy depots, including fat and protein, are mobilized to provide circulatory glucose. The ultimate fate of metabolizable carbohydrate is oxidation, whereby it is converted to energy, carbon dioxide, and water. Thus, the original intrinsic energy of the sugar molecule is made available for various biological functions, and any excess energy is dissipated as heat.

The total energy of a carbohydrate molecule has been determined by thermal combustion in a bomb calorimeter. By this means it is now known that carbohydrates provide the same number of Calories as do proteins, i.e., about one-half the Calories of the fat components of the diet. The large Calories per gram of food substance, adjusted for their coefficient of utilization, are carbohydrate, 4; protein, 4; and fat, 9. An equation relating dietary composition to calorific value is:

$$\text{Cal/100 gm} = 4 \times \text{Wt Carbohydrate} + 4 \times \text{Wt Protein} + 9 \times \text{Wt Fat}$$

The Calories ingested by the human are related to the organoleptic effects produced. These, in turn, govern the signal to the brain to suggest appetite or aversion. Dietary sugars that are absorbed with difficulty give rise to gastrointestinal disturbance.

TASTE

Sweetness

In addition to an intrinsic energy attribute, the food sugars usually possess the important intrinsic sensory attribute of sweet taste. It is a logical deduction, since paraffinic molecules are not sweet, that the sweetness of the sugars is a function of the hydroxyl groups, or of the hydroxyl groups and the ring oxygen atom. However, polyhydroxy compounds vary so much in sweetness that it is clearly not only the existence of polyhydroxyl groups as such that governs sweetness. β-D-Glucose is sweet, but its epimer, β-D-mannose, is mostly bitter. Furthermore, many amino acids, some metallic salts,

and unrelated compounds such as saccharin and chloroform elicit the same sensation.

Elucidation of the reasons why sugars taste sweet, and why they vary in their sweetness, interrelates all aspects of their structure with this sensory attribute. As an example, it was recognized early in the study of sugar structures (Wells 1858) that the molecular formula, $C_6H_{12}O_6$, was not entirely satisfactory since "some hexoses are sweet, others slightly sweet, and still others are destitute of sweetness." Table 5.1 lists some of the milestones among the many

TABLE 5.1

HISTORICAL DEVELOPMENT OF THEORY RELATING THE STRUCTURE OF COMPOUNDS TO SWEET TASTE

Investigators	Year	Observation or Theory
Cohn	1914	Saporous groups occur in pairs
Oertly and Myers	1919	The auxogluc and glucophore requirement
Kodama	1920	"Vibratory hydrogen"
Tsuzuki	1948	Resonance energy
Deutsch and Hansch	1966	Hydrophobicity and the Hammet σ constant
Shallenberger and Acree	1967	The AH,B couple and hydrogen bonding
Kier	1972	AH,B plus a "dispersion" requirement

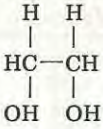
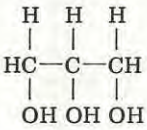
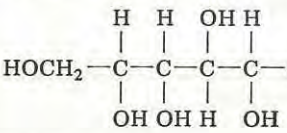
studies devoted to explaining the relation between chemical structure and function and sweet taste. Cohn (1914) recorded the observation that a multiplicity of hydroxyl groups usually results in sweet taste, and that saporous groups seem to occur in pairs. Oertly and Myers (1919) characterized the two saporous groups as an "auxogluc" and a "glucophore," by analogy with the then current theory of an auxochrome and a chromophore needed for dye color. Some of their auxoglucs and glucophores are shown in Table 5.2.

Kodama (1920) and Tsuzuki (1948) were concerned primarily with the energetics of sweet-tasting compounds, and Deutsch and Hansch (1966) pointed out that in a closely related series of compounds, such as the 2-amino-4-nitrobenzenes, relative sweetness correlated well with their hydrophobicity.

Shallenberger and Acree (1967) developed the idea of the AH,B couple as the unit prerequisite to sweet taste in compounds; for the sugars, the AH,B unit had to meet rigid stereochemical requirements. To the AH,B couple, Kier (1972) added a third element described as a dispersion unit, which seems to be related to sweetness intensity.

TABLE 5.2

AUXO- AND GLUCOPHORIC COMPONENTS OF SWEET-TASTING ACYCLIC COMPOUNDS

Compound	Structure	Glucophore	Auxogluc
Glycol		$CH_2OH—CHOH$	H
Glycerol		$CH_2OH—CHOH$	CH_2OH
Glucose		$—COC—HOH—(H)$	H
Glycine	$CH_2(NH)_2CO_2H$	$CH(NH)_2CO_2H$	H
Chloroform	$CHCl_3$	CCl_3	H

Source: Oertly and Meyers (1919).

We describe this third site as γ; it incorporates Deutsch and Hansch's idea of hydrophobicity within a structural requirement for sweet taste intensity. The stereorelation between these three sites required to elicit sweet taste is described by Shallenberger as "multiple group stereogeometry" (Birch and Shallenberger 1973).

The approximate sweetness scores for various sugars are shown in Table 5.3. The variance in the range given for the sweetness of a sugar is due to a number of factors. In solution the rapid mutarotation of fructose to give a mixture of tautomers that differ widely in their sweetness yields scores that are rather high for freshly dissolved material, and low for the equilibrium mixture. Since no instrumental method has been devised for the measurement of sweetness, a taste panel determination must be utilized (Spencer 1971). The variation in scores using different panel techniques is also one of the reasons for the varying sweetness scores found in the literature (Shallenberger and Acree 1971).

No explanation of the phenomenon of sweetness based on the known ring structures of the sugars was offered until 1963. It was recognized (Shallenberger 1963) that since sweetness depended on

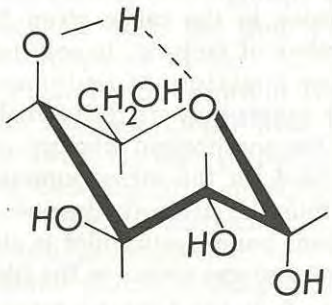
TABLE 5.3

THE RELATIVE SWEETNESS OF SUGARS

Sugar	Sweetness	
	In solution	As "Crystalline" Material
β-D-Fructose	100–175	180
Sucrose	100	100
α-D-Glucose	40–79	74
β-D-Glucose	<α-D-anomer	82
α-D-Galactose	27–27	32
β-D-Galactose		21
α-D-Mannose	59	32
β-D-Mannose	bitter	bitter
α-D-Lactose	16–38	16
β-D-Lactose	48	32
β-D-Maltose	32–46	
Raffinose	23	1
Stachyose		10

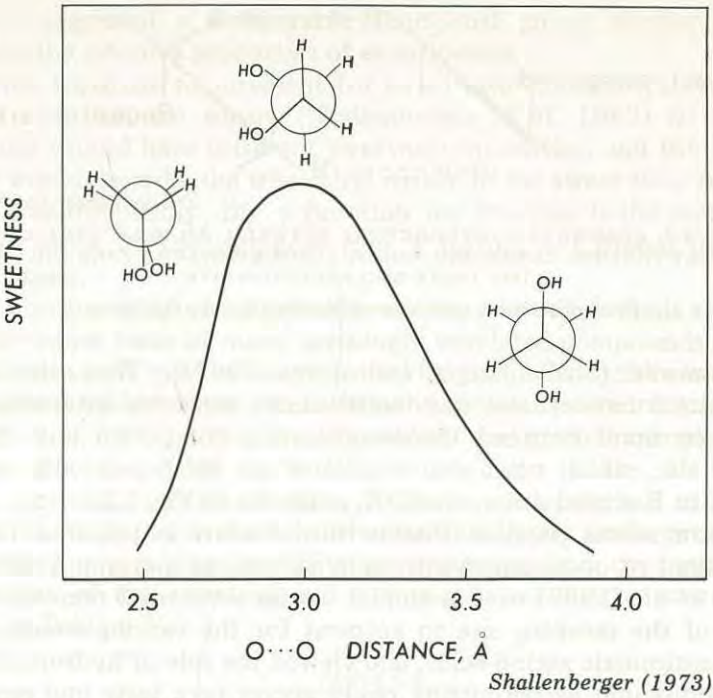
Source: Shallenberger and Acree (1971).

concentration, temperature, and α-glycol hydroxyl groups and their relative disposition, the entire phenomenon of sweetness may be mediated by hydrogen bonding. Studies of molecular models of the sugars indicated that certain conformations of the α-glycol groups can lead to intramolecular hydrogen bonding between hydroxyl groups, while others cannot. Furthermore, certain stereodispositions of hydroxyl groups, such as the axial hydroxyl group on carbon atom number 4 of galactose, and on carbon atom number 2 of mannose, can hydrogen-bond the ring oxygen atom (Barker *et al.* 1959).



α-D-GALACTOPYRANOSE

Characteristic arrangements of the α-glycol group in the sugar ring, as related to sweetness potential, are shown in Fig. 5.1. In



Shallenberger (1973)

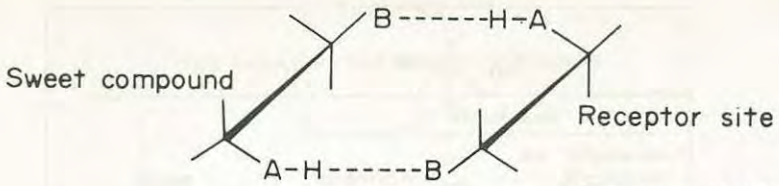
FIG. 5.1. α-GLYCOL CONFORMATION OF SUGAR RING STRUCTURES AND THEIR APPROXIMATE POTENTIAL FOR ELICITING SWEET TASTE

Reproduced with permission of the American Chemical Society.

conformational terms, either an axial-equatorial (*cis*) or di-equatorial (*trans*) disposition of OH groups provides the *gauche* or staggered glycol conformation, and is capable of eliciting sweet taste unless one of the OH groups is sterically disposed to bond to the ring oxygen atom. In the *gauche* arrangement, the centers of each oxygen atom in the α-glycol unit are separated by 2.86Å, which is just beyond the limit for significant intramolecular hydrogen bonding to occur (Pauling 1960).

Vicinal OH groups in the diaxial (*trans*) anti glycol conformation could not be related to sugar sweetness, since they are too far apart to permit intramolecular hydrogen bonding. Vicinal OH groups in the diaxial (*cis*) eclipsed conformation establish the system for the strongest intramolecular hydrogen bond.

By viewing the α-glycol group in the *gauche* conformation as an AH,B system used to define and describe the hydrogen bond, it was deduced that the average AH proton to B orbital distance of 3Å was the steric requirement for sweet taste mandated by the



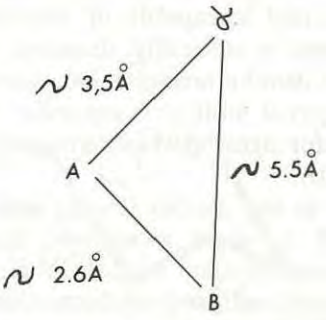
Shallenberger (1971)

FIG. 5.2. CONCERTED INTERACTION BETWEEN AN AH,B UNIT OF A SWEET-TASTING COMPOUND AND A COMPLEMENTARY AH,B UNIT AT THE TASTE BUD RECEPTOR SITE

Reproduced with permission of Applied Science Publishers.

receptor site (Shallenberger and Acree 1967). Thus, the initial chemistry for sweet taste was described as a concerted intermolecular hydrogen bond between the sweet-tasting compound and the receptor site, which must also consist of an AH,B pair with an AH proton to B orbital distance of 3 Å, as shown in Fig. 5.2.

It now seems possible that a third feature is required for the attainment of optimum sweetness in a chemical compound. Shallenberger *et al.* (1969) used a spatial barrier somewhat removed from AH,B of the receptor site to account for the varying sweetness of the enantiomeric amino acids, and viewed the role of hydrophobicity of a compound as permitting ready access to a taste bud receptor site due to the lipoid nature of biological membranes. Kier (1972), however, pointed out that a third feature required for sweet taste is a dispersion, or hydrophobic site, located about 3.5 Å from atom A of AH, and about 5.5 Å from B. This relation is shown below, using γ to depict the hydrophobic ("greasy") site:



The interesting feature of Kier's proposal, which we take the liberty to point out, is that the tripartite grouping of AH,B and γ is dissymmetric, since the distance parameters describe an oblique triangle not superposable upon its mirror image. Shallenberger

(1973) suggested a comparable functional group relationship to explain the odorous properties of enantiomers.

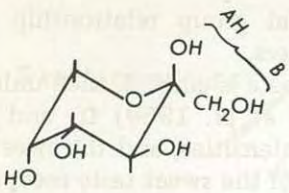
If the tripartite requirement for sweet taste is correct, then unlike other enantiomeric sugars (Shallenberger *et al.* 1969) D- and L-fructose should have different sweetness intensities, and the sweeter sugar would describe the true chiral nature of the sweet taste receptor site. Parenthetically, the γ function for fructose is the methylene carbon atom of the pyranose ring, a feature not shared by other food sugars.

As confirmation of its probable validity, the AH,B thesis accounts for the sweet taste of many seemingly unrelated compounds such as chloroform and salts of beryllium (Fig. 5.3). Artificial sweeteners are often hundreds or thousands of times sweeter than sugars (Birch *et al.* 1971). This fact is probably due to the hydrophobicity of the structures and the relative inflexibility of the AH,B unit. Thus saccharin and the 2-amino-4-nitrobenzenes are not subject to steric or tautomeric change, whereas the sugars undergo a time-dependent conversion to different tautomers and conformations. Examples of the sweetness of compounds relative to sucrose are given in Table 5.4.

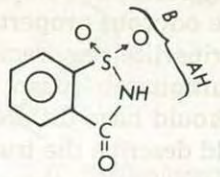
TABLE 5.4

SWEETNESS OF VARIOUS SUBSTANCES RELATIVE TO SUCROSE

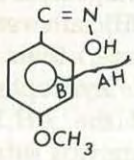
Substance	Relative Sweetness
Glycerol	0.5
Sorbitol	0.5
Sucrose	1.0
2-Methylcyclohexylsulfamate	15
p-Anisylurea	18
Sodium cyclohexylsulfamate (sucaryl)	15–31
Chloroform	40
Methoxy-2-amino-4-nitrobenzene	167
p-Methylsaccharin	200
p-Phenetylurea (dulcin or sucrol)	70–350
6-Chlorosaccharin	100–350
Saccharin	240–350
n-Hexylchloromalonamide	300
Stevioside	300
2-Amino-4-nitrotoluene	300
p-Nitrosuccinanilide	350
p-Methoxymethylnitrobenzene	500
1-Bromo-5-nitroaniline	714
*p*erillaldehyde *anti*oxime (perillartine)	2,000
n-Propoxy-2-amino-4-nitrobenzene (P-4,000)	4,000



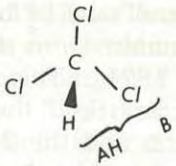
β –D FRUCTOSE



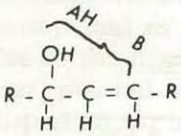
SACCHARIN



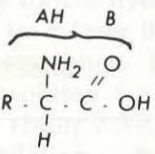
∝ - ANISALDEHYDE OXIME



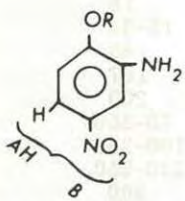
CHLOROFORM



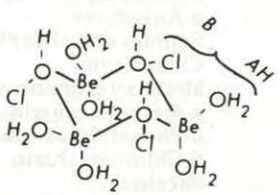
UNSATURATED ALCOHOL



∝ - AMINO ACID



2 - AMINO - 4 - NITROBENZENE



BERYLLIUM HYDROXO - CHLORIDE

Shallenberger and Acree (1969)

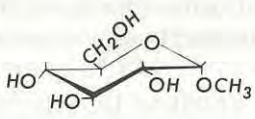
FIG. 5.3. REPRESENTATIVE COMPOUNDS WHICH TASTE SWEET AND THE AH,B UNIT COMMON TO ALL OF THEM

Reproduced with permission of the American Chemical Society.

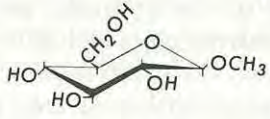
Further evidence of the correct assignment of the AH,B location in the compounds illustrated in Fig. 5.3 can be obtained by single substitution at AH or B. For example, synthesis of the interesting compound N-β-D-glycosyl saccharin (Acton *et al.* 1968) resulted in the complete loss of sweetness, the product being intensely bitter.

An implication of the AH,B theory is that, although the molecular geometry of the different glycol groups varies, it is probable that only one such group will be geometrically optimum for binding to the taste bud protein. Location of the AH,B system within sugar molecules presents an intriguing task, whose success depends on selection of a sufficient number of suitable model analogues (Birch and Lee 1971).

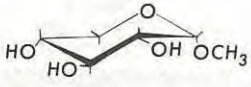
A highly reactive anomeric center is not a prerequisite of sweetness, since the nonreducing sugar sucrose and α,α-trehalose are sweet. For these compounds, the AH of the AH,B system is not at the anomeric center, and must reside at some other point in the molecule. Because reducing sugars isomerize in solution, investigations into the finer chemical basis of sugar sweetness should perhaps be done with glycosides. In substituting the anomeric center, however, the difficulty that glycosides and other model substituted sugars may have an increased bitter taste may arise. Of the 4 possible methyl glycosides of glucose and xylose, for instance, 3 have a mixed sweet and bitter taste, and only methyl α-D-glycopyranoside is not bitter.



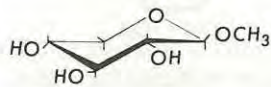
METHYL α-D-GLUCOPYRANOSIDE
(SWEET)



METHYL β-D-GLUCOPYRANOSIDE
(BITTER-SWEET)



METHYL α-D-XYLOPYRANOSIDE
(BITTER-SWEET)



METHYL β-D-XYLOPYRANOSIDE
(BITTER-SWEET)

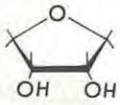
Bitterness may very well be imparted by the methoxyl substituent in some configurations, but some free β-anomers of the sugars, such as β-D-mannose, are also bitter. Bitterness, therefore, appears to be imparted by a combination of effects involving the configuration of the anomeric center, the ring oxygen atom, the primary alcohol

group of hexoses, and the nature of any substituents. Since bitterness is known to depress sweetness (Birch *et al.* 1973), it is clearly important to eliminate such contaminating effects in the selection of model sugars for studying the stereochemical basis of sweetness.

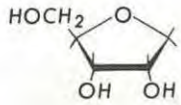
Contaminating tastes due to anomeric substituents, as described above, can be avoided by using nonreducing oligosaccharides and their derivatives as models. The limited number of such sugars and their asymmetric nature are drawbacks in modifying them for sensory evaluation. Only one nonreducing oligosaccharide appears to be uniquely suitable as a model in this context, namely, the di-saccharide α,α-trehalose. The absolute structure of this sugar is now known (Birch and Richardson 1968). It is an extremely stable sugar having a twofold axis of symmetry. Sensory evaluation of a large number of derivatives of α,α-trehalose and its analogs indicates which hydroxyl groups are not of any particular significance in the AH,B system. Such studies have shown (Birch *et al.* 1971) that the fourth hydroxyl group of glucopyranoside structures appears to be of special importance, possibly constituting the AH of the AH,B system.

Location of the AH,B system in sugar structures may not yield the same result in different biological species. However, Evans (1963), working with the blowfly, discovered that the fourth hydroxyl group of the sugar pyranose structure is involved in binding to the taste bud protein. It can be concluded from these results that many of the hydroxyl groups and other structural features of sugar derivatives are surplus to the structural requirements needed to elicit sweet taste response.

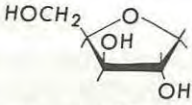
Simple sugar analogues may provide excellent models for sensory evaluation. Most of these are expectedly sweet:



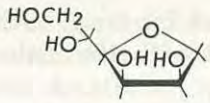
1,4-ANHYDROERYTHRITOL
(NOT SWEET)



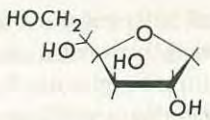
1,4-ANHYDRORIBITOL
(SWEET)



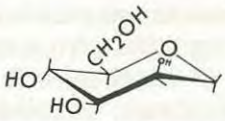
1,4-ANHYDROXYLITOL
(SWEET)



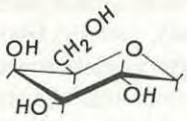
1,4-ANHYDROMANNITOL
(SWEET)



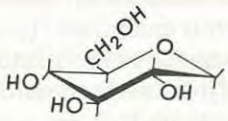
1,4-ANHYDROGLUCITOL
(SWEET)



1,5-ANHYDROMANNITOL
(SWEET)

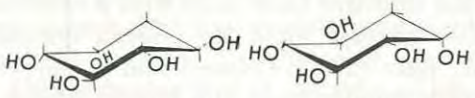


1,5-ANHYDROGALACITOL
(SWEET)



1,5-ANHYDROGLUCITOL
(SWEET)

The cyclitols (quercitols and inositols) are simple structural analogs of the sugars that do not mutarotate, since they have neither a ring oxygen atom nor an anomeric center. The racemate, DL-viboquercitol, for example, is at least as sweet as sucrose. The D-enantiomer is an analogue of α-D-glucose with a methylene group replacing the ring oxygen atom, whereas the L-form is not a simple analog of any of the common sugars. When L-viboquercitol was synthesized, it was found to be devoid of sweetness (Birch and Lindley 1973). The D-enantiomer is at least twice as sweet as sucrose:

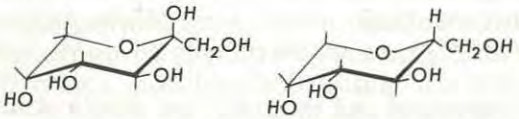


L-VIBOQUERCITOL D-VIBOQUERCITOL

This relatively high sweetness supports the idea that surplus structural moieties can be removed from sugar molecules, with a corresponding advantage in sweetness. However, most of the di-, tri- and tetrahydroxy cyclohexanes so far evaluated are devoid of sweet taste; thus it is possible that some of the hydroxyl substituents in ring structures, though not specifically involved on the active site, may be participating in some allosteric manner. Although a number of the cyclitols have now been evaluated and several are analogs of well-known α- and β-anomers of the sugars, none of them exhibits

any trace of bitterness. This provides support for the idea that the ring oxygen atom is in some way involved with the bitter taste response.

The particular significance of the fourth hydroxyl of pryanose structures, elucidated after sensory evaluation of the disaccharide α,α-trehalose and its derivatives, may no longer apply when the conformation of the sugar ring differs from the usual Cl type. Fructopyranose, for example, is the sweetest sugar known and exists in the alternative 1C conformation. Shallenberger and Acree (1967) suggested that the 1,2 glycol group constitutes the saporous AH,B unit in this substance, and in accordance with this idea the analog 1,5-anhydro-D-mannitol (1-deoxy mannose), which is the same compound as 2-deoxy fructopyranose, though sweet, is considerably less so than β-D-fructopyranose itself:



β-D-FRUCTOPYRANOSE 2-DEOXY-D-FRUCTOPYRANOSE
OR
1-DEOXY-D-MANNOPYRANOSE

The Primary Sensation of Sweetness and its Qualitative Assessment

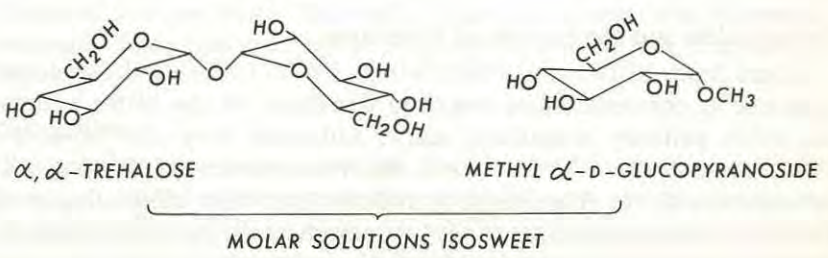
The 4 primary taste sensations—salty, sour, sweet, and bitter—are principally located on different areas of the tongue. Salty and sour tastes occur mainly at the sides of the tongue, whereas bitterness occurs at the back and sweetness near the tip of the tongue. Stimulation of the sensitive taste buds with a sweet substance causes electrical impulses to pass along the chorda tympani nerve to the brain. However, Kare (1969) showed that a radioactive sugar solution may pass very rapidly from the mouth of the rat directly to the brain (presumably by vascular action) without passing down the esophagus to the stomach. Thus the phenomenon of sweetness may be the end result of a highly complicated set of interacting physiological functions.

Each taste bud consists of about fifty taste cells which are subject to almost constant stress from the variety of different chemicals that arrive on the tongue. They are eventually destroyed, but are also replenished by mitotic division. The life of each cell is only about ten days (Beidler 1971). The AH,B theory of sweetness, based on stereochemical explanations of the phenomenon, also seems justified on the ground that the primary stimulus is probably caused by direct

action of the saporous substance on the receptor protein of the taste cell membrane. If such proteins could be removed from the tongue without denaturing them, it should be physically possible to measure the strength of binding between them and the sugars, and to relate this to sweetness. Dastoli and Price (1966) have already attempted this, and have claimed success in measuring the strength of binding of the complex between various sugars and the protein isolated from bovine tongues. The strength of binding was determined as the effect on the protein of change in refractive index or U.V. absorption. This work has not been substantiated by other investigators, nor has any instrumental method for determining sweetness been devised.

All measurements of sweetness and relative sweetness recorded in the literature have been obtained with taste panels. These must be pre-selected to ensure that the panelists are sufficiently sensitive to sweetness to be able to rank samples in the correct order. Several workers have reported that subjective intensity of sweetness (an arbitrary score awarded by panel members) is directly proportional to the log of the concentration of the sweet substance (Schutz and Pilgrim 1957; Birch *et al.* 1970, 1973). However, Beidler (1971) states that the magnitude of depolarization of the taste nerve cell is hyperbolically related to the concentration of the sweet substance. These differences may be due to lack of experimental precision by the very nature of the subjective response. In any case, these mathematical relationships are not yet understood in terms of the nature of binding of the saporous molecule to the taste bud.

If a trained taste panel is allowed to repeat a ranking experiment a number of times, statistically reliable results can be achieved. In this way it was found that the model sugar α,α-trehalose was isosweet with its analog, methyl α-D-glucoside, on a molar basis (Birch *et al.* 1970). This indicates that if the type of binding postulated in the AH,B thesis is operating, then only one-half of the trehalose molecule is binding to the taste bud protein:



This conclusion had already been anticipated because of the well-known usual decrease in sweetness with increasing molecular weight of carbohydrate; therefore, raffinose and maltotriose have little more than a trace of sweetness, and the tetrasaccharides, with the exception of stachyose, may have none.

It has already been mentioned that many rare food sugars and their derivatives are bitter, and that bitterness depresses sweet taste. This depression can be expressed on a mathematical basis by the equation:

$$\Delta T = K \log C/C_m$$

where

ΔT = depression of sweetness intensity produced by a bitter additive;

C = concentration of bitter additive producing depression of sweetness ΔT;

C_m = maximum concentration of additive having no effect;

K = a constant which can be determined for a family of related compounds such as the sugars.

Using this formula, it is possible to calculate the depression in sweetness of a particular rare food sugar due to its own intrinsic bitterness (Birch *et al.* 1973). This technique has been used to investigate the effect of the anomeric configuration on sweetness response. Thus, although conflicting reports have been published about the relative sweetness of α- and β-D-glucose, the available evidence suggests that the anomeric center is not of much significance in binding to the taste bud protein. However, methyl-α-D-glucopyranoside, which is not bitter, appears to be about 50% again as sweet as methyl-β-D-glycopyranoside, which is also bitter. When the bitterness is measured by taste panels, and the formula shown above is utilized, it is found that the difference in sweetness between the two substances can largely be accounted for.

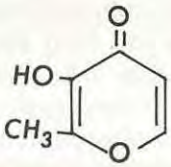
Potentiation and Depression of Sweetness

Apart from bitterness, which is effective in lowering the sweetness response at concentrations near the threshold of the bitter additive, the other primary sensations, salty, and sour, may also be able to lower sweetness. Furthermore, the temperature of the saporous substance and its rheological condition can also affect the result. Possibly macromolecules such as starch and its derivatives and carboxymethylcellulose can affect the result by occupying active

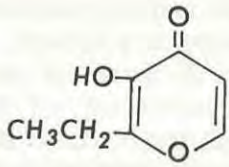
or allosteric sites on the taste bud protein, or by altering the activity of the saporous AH,B unit itself.

Complex psychological interactions can result from the masticatory influence induced by dilatory and thixotropic substances. Simple sugars themselves, such as sucrose, can contribute to this by exerting thickening and bodying action in the mouth. Most sugars, when mixed, have a synergistic effect on one another's sweetness, so that foods such as honey have an enhanced sweetness.

Flavor enhancers can also be used to achieve this effect, the best known of these being maltol and its ethyl analog.



MALTOL



ETHYL MALTOL

Maltol can enhance sweetness and cause an apparent change in texture to that of a more "velvety" sensation. The derivative isomaltol is 6 times as effective as maltol as a sweetness enhancer, but it is not known whether either substance functions by making the receptor protein more available to the saporous molecules or by nerve activation. Maltol lowers the threshold concentration of sucrose by a factor of 2 (Birch 1973); hence, if the receptor sites are not occupied below the threshold concentration, the latter of these two possibilities cannot be true.

Production and Abolition of Sweetness

The tropical fruit known as the miracle berry (*Richardella dulcifica*) has the property of making sour substances taste sweet. The effect, which is caused by a glucoprotein termed miraculin, lasts for some hours. Sweetness can be obliterated by administration of the active principle of the plant *Gymnema sylvestre*, so that crystals of sucrose taste like sand. The active principle, known as gymnemic acid, has not been properly characterized, nor has its effect been explained.

Aglycogeusia

Patients have been described by Henkin and Shallenberger (1970) who are unable to recognize the taste of any sweet substances, although their ability to respond to the primary sensations salty, sour, and bitter are normal. These patients were termed aglyco-

geusics (Gr. *glykys*, sweet; *ageusia*, without taste); with them fructose and sucrose usually elicited a sour taste response, while glucose and galactose usually elicited a bitter response. The only pentose tested was D-xylose, which usually elicited a salty response.

DIGESTION OF CARBOHYDRATES

The digestion or enzymic breakdown of carbohydrates appears to be a normal initial requirement that precedes their absorption, although some evidence has been produced for the absorption of intact sucrose and maltose molecules. Enzymes therefore normally hydrolyze oligo- and polysaccharides to monosaccharides in various areas of the alimentary system. These are then absorbed through the intestinal wall from the mucosal side into the blood stream (serosal side).

Historically it was believed that phosphorylation played an important part in the digestion and absorption of carbohydrates both by the degradative enzyme, phosphorylase, and the absorption of phosphorylated forms of the sugars such as glucose-1-phosphate or fructose-6-phosphate. Whether or not the enzymes were particulately bound was a matter of some dispute, many textbooks stating that digestion takes place in the lumen of the intestine by the action of a secretion known as the succus entericus. In fact, enzyme digestion is initiated by an amylase present in the saliva, but this is arrested by acidity when the food reaches the stomach. Subsequently when the food mass reaches the small intestine, the alkaline pH allows a secretion from the pancreas (pancreatic amylase) to act on starches hydrolyzing them to a mixture of low molecular weight carbohydrates, maltose being the major product.

The resultant mixture of low molecular weight sugars undergoes completion of hydrolysis by the action, mainly, of particulately bound glycosidases in the brush border region of the small intestine. Finally, the resulting monosaccharides are absorbed through the intestinal wall in one of three ways:

(1) passive diffusion (slow absorption by osmotic pressure),
(2) facilitated diffusion (the sugar is absorbed at a faster rate than can be explained by simple diffusion),
(3) active absorption (the sugar is absorbed against a concentration gradient).

Mannose is an example of a sugar absorbed only by passive diffusion and is thus, recognized as a very poorly absorbed sugar. Fructose is absorbed considerably faster than mannose by virtue of

facilitated diffusion. Glucose and galactose are actively absorbed sugars that are able to accumulate on the serosal side of the intestinal wall against a concentration gradient.

The particulately bound enzymes of the brush border are located on the microvilli, and there are two galactosidases (lactases) and five glucosidases. The latter are all nominally maltases, as they hydrolyze maltose, but in addition certain of them hydrolyze other sugars such as isomaltose, sucrose, and trehalose. The function of trehalase is still obscure, since trehalose does not constitute a significant part of the diet. This enzyme will be discussed further. It is clear that this diverse mixture of glycosidases can cope with starch and its decomposition products (maltodextrins).

Active Absorption

Crane (1968) suggested a model for the active absorption of sugars to replace the currently held view that phosphorylation is an essential intermediate step in the absorption process, which was based on evidence which conflicted with the phosphorylation theory. For example, the compound 3-*O*-methyl-D-glucose was actively absorbed, but almost certainly not phosphorylated.

It was suggested that an actively absorbed sugar is attached to a "carrier" protein on one side of the transport cell membrane and then transported to the other (serosal) side of the membrane where the carrier gave up the sugar molecule and returned to the mucosal side to pick up more sugar. This model accounts for the net transfer of sugar from a region of lower to a region of higher concentration, and appears to be mediated by sodium ions, as shown in Fig. 5.4. It is characteristic of animal cell membranes that they contain an energy-dependent outwardly directed "sodium pump," which returns sodium ions to the medium in which they are suspended. The affin-

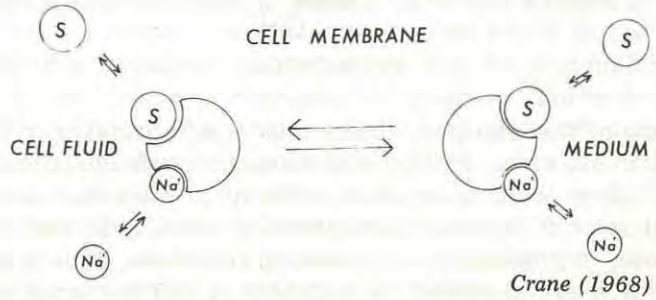
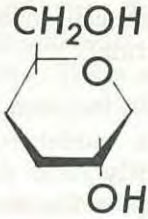


FIG. 5.4. TRANSPORT OF A SUGAR ACROSS A BIOLOGICAL MEMBRANE

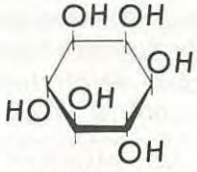
Reproduced with permission of Academic Press.

ity between the sugar and carrier appears to be dependent on the concentration of sodium ions. The higher the concentration of sodium ions the greater the affinity; the sodium thus functions in an allosteric manner possibly, as depicted in Fig. 5.4. The other alkali metal ions K^+, Li^+, Ca^+, and also NH_4^+ inhibit sugar transport.

According to Crane (1968) the basic structural requirement for active sugar transport is a pyranose structure with a primary alcohol group at position 5 and a second hydroxyl group at the second carbon atom with the same configuration as found in D-glucose:



Thus the pentoses such as xylose are not actively absorbed because they do not possess a primary alcohol group. D-Mannose is very poorly absorbed because it does not have the appropriate configuration at the second carbon atom. However, L-glucose, which has both the configurational and conformational mirror-image structure of D-glucose, also undergoes active absorption. More work by Crane (1970) indicates that the isomer, myoinositol, also undergoes active absorption but by a different system from the sugars.



MYOINOSITOL

A study of the transport characteristics of a number of different sugars (Barnett *et al.* 1970) using hamster small intestine revealed that 6 of them were transported actively at rates that were in the following order of decreasing activity: L-glucose, D-fucose, D-xylose, L-arabinose, myoinositol; L-fucose, L-rhamnose, and D-mannose were not actively absorbed by segments of hamster small intestine. The moderately fast absorption of L-arabinose is interesting as it differs from the very quickly absorbed D-galactose only in the absence of the primary alcohol group.

Although chemical modification at one or more hydroxyl groups may affect the rate of intestinal absorption, it does not usually eliminate it. However, it does appear that the D-gluco configuration at position 2 remains an absolute requirement if the sugar does not possess a primary alcohol group. If it does, the opposite configuration may be actively absorbed. All of the hydroxyls of a sugar may bind to the carrier protein, but, despite the stereostructural significance of the second, it is the third hydroxyl which may be linked the most strongly as a covalent ester-type of bond (Barnett *et al.* 1970).

Blood Glucose

Immediately after passage of a sugar through the intestinal wall, whether by passive or facilitated diffusion or by active absorption, it appears in the bloodstream as blood sugar. The normal active absorption process is so rapid that the concentration of sugar in the blood would reach an intolerably high level were it not controlled in some way. This control is effected by the hormone insulin. Insulin is released from the beta cells of the pancreas in response to the presence of carbohydrate and it maintains the blood sugar level within well-defined limits. In a normal person the blood sugar level may be fairly constant at about 80 mg per 100 ml after a period of fasting, but will rise to about 150 mg per 100 ml in less than an hour after the consumption of a meal. In diabetics, however, who suffer from a deficiency of the hormone insulin, the fasting level may be near the peak level for normal people and reach 320 mg per 100 ml or higher after a meal (Fig. 5.5). This result is usually accompanied by a high level of glucose or other sugar in the urine.

Most of the sugar entering the bloodstream from a normal diet is derived from starch or sucrose, so that normally at least 70 to 80% of it enters the bloodstream as glucose. A certain amount of fructose (mostly from sucrose) and possibly any rare sugars in the diet will also enter the bloodstream, and these may be metabolized quite quickly in the liver and converted to glucose. Therefore, after a period during which the concentration and type of sugar in the circulation is adjusted and controlled by the action of the liver and the hormone insulin in particular, all the circulating sugar can be considered to be glucose. Insulin may convert excess glucose to glycogen and/or oxidation products such as lactate. Rare food sugars may be excreted in the urine. It is not certain to what extent the release of insulin is affected by the actual concentration of glucose in the blood and/or the presence of carbohydrate in the intestine. There is evidence in support of both these possibilities.

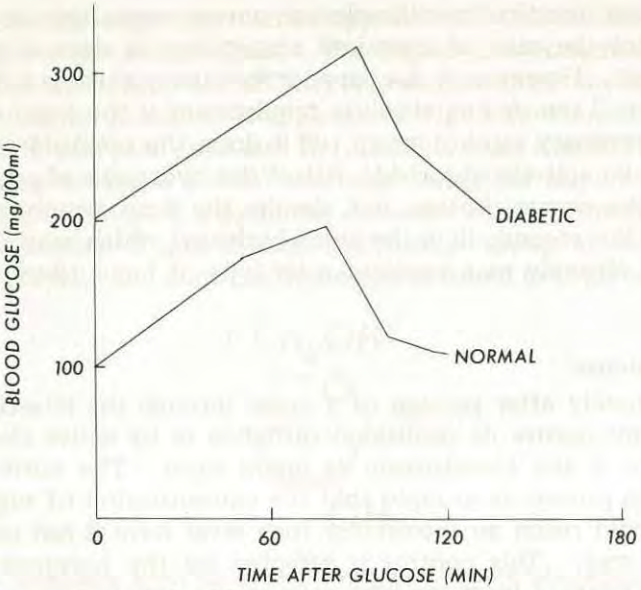


FIG. 5.5. TYPICAL GLUCOSE TOLERANCE CURVES FOR NORMAL AND DIABETIC SUBJECTS

Furthermore, insulin release is also governed by psychological factors. For example, when subjects in a fasting state were told under hypnosis that they were consuming a large meal, their insulin levels were observed to rise (Goldfine *et al.* 1970).

It can be assumed that all amylose, amylopectin, and maltodextrins in the diet can be adequately hydrolyzed to low molecular weight sugars, such as glucose, maltose, isomaltose, etc., as a result of the presence of intestinal and pancreatic amylase. But the entire roles played by these enzymes, as well as by alkaline phosphatase that may be fundamentally involved in the digestive process in hydrolyzing sugar-phosphate ester bonds, are not altogether clear. There is evidence that the amylases, whether intestinal or pancreatic in origin, vary in significance with the age of the individual and specificity of the enzyme. During the first year of infancy, for instance, intestinal amylase, which has a high specificity for low molecular weight maltodextrins such as maltotetraose, may be important because this sugar constitutes a significant part of the carbohydrate in the intestine (Alpers and Solin 1970). Furthermore, the rate at which a particular carbohydrate in the diet raises the blood sugar level is not always predictable on the basis of molecular weight. Some mixtures of maltodextrins raise the blood glucose level faster

than dextrose itself. Fructose, which is absorbed more slowly (by facilitated diffusion) than D-glucose and the maltodextrins, is of possible value to mild diabetics.

RARE FOOD SUGARS, GLUCOSE SYRUP, MALABSORPTION, AND OTHER ILL EFFECTS

Rare Food Sugars

In all normal diets in which the carbohydrate consists of starch and sucrose, with a small quantity of lactose (milk sugar), the enzymic hydrolytic systems outlined above are usually able to digest these carbohydrates in the intestine. It can be seen, however, that even minor structural alterations in the carbohydrate molecules (e.g., D-glucose and D-mannose, which differ only at C-2) can drastically affect the way the body is able to deal with them.

Rare food sugars have been defined (Birch 1969) as those food sugars that do not normally occur to any significant extent in the diet. The alimentary system has not evolved the enzymes needed to deal with them digestively, and hence malabsorption syndromes and other gastrointestinal disturbances may result. The importance of the rare food sugars lies in possible increase in their use because of the advance of modern technology. Some of the better-known rare food sugars are listed in Table 5.5, and those with reported physiological effects in Table 5.6.

The most obvious result of malabsorption of a particular sugar is that it may pass slowly into the gut wall, causing water absorption effects and osmotic diarrhea. Any part of the sugar that lingers in the intestinal lumen is liable to attack by gut microorganisms, which

TABLE 5.5

TYPES OF RARE FOOD SUGARS OR THEIR DERIVATIVES

Monosaccharides	Disaccharides	Trisaccharides	Tetrasaccharides
Ribose	Lactose	Maltotriose	Stachyose
Arabinose	Maltose	Panose	
Xylose	Isomaltose	Raffinose	
Xylitol	Gentiobiose		
Sorbitol	Trehalose		
Levoglucosan	Cellobiose		
Mannose	Neohesperidose		
Mannitol			
Rhamnose			
Galactose			
Maltol			
Ethyl Maltol			

TABLE 5.6

RARE FOOD SUGARS AND PHYSIOLOGICAL ILL EFFECTS

Sugar	Malabsorption	Flatulence	Diarrhea	Cataract
Xylose	?	—	+	+
Arabinose	—	—	—	+
Galactose	—	—	+	+
Sorbitol	—	—	+	—
Maltose	+	+	+	—
Isomaltose	+	+	+	—
Lactose	+	+	+	+
Raffinose	?	+	—	—
Stachyose	?	+	—	—

+ Literature reports of effects.
– No literature reports or poorly substantiated reports.

convert it to acids that in turn have a scouring effect in the intestine, producing frothy, evil-smelling stools and possibly more severe gut damage, including permanent lesions and even tumors.

D-Xylose (wood sugar) and its hydrogenated derivative D-xylitol produce the predictable diarrhea symptom in rats and probably also in human beings if consumed in sufficient quantities. Even sorbitol, which is widely used in diabetic chocolate in the United Kingdom and United States, has a mild laxative effect in moderate quantities and will produce diarrhea in large quantities. Before causing diarrhea, rare food sugars may induce excessive flatulence, again by action of the gut microorganisms. The tetrasaccharide, stachyose, which occurs in the soya bean and the U.S. navy bean, is believed to be active in this respect, causing cramping pains, borborygmia and associated intestinal discomfort.

Work carried out in recent years at National Aeronautic and Space Administration (Shapira 1967) has been aimed at converting human waste products into utilizable carbohydrate. One of the simplest substances to be obtained from human waste products is formaldehyde. When formaldehyde is treated with alkali it is converted to a mixture of tetroses, pentoses and hexoses known as "formose"; the mixture, however, is found to be very toxic to rats, probably because of the pentose content. Glycerol can also be obtained from human waste products, and this substance appears to be quite safe when fed to rats in high concentrations.

Another of the effects listed in Table 5.6 is cataract, which has been observed only in laboratory animals (chiefly rats of a certain strain) and has never yet been demonstrated in humans as a result of rare food sugar consumption. Cataractogenic sugars (mainly galactose, lactose, and xylose) appear to cause a series of oxidative-

reductive biochemical changes; the primary toxin, which is the sugar alcohol galactitol (or xylitol), accumulates in the lens of the eye. This then instigates deposition of insoluble protein fibers in the lens, which causes the opacity (van Heyningen 1969). It is interesting that the sugar alcohols themselves cannot cause cataract because they cannot penetrate the membrane of the lens. It is also interesting that L-arabinose, which differs from D-galactose only in the absence of the primary alcohol group, can enhance the cataractogenic effect of other sugars but apparently cannot cause it itself. An experiment in which rats were fed solely on a diet of yogurt (which contains a high proportion of lactose) for a few weeks resulted in a 100% incidence of cataract (Richter and Duke 1970).

Trehalose and Trehalase.—The rare food sugar α,α-trehalose (mushroom sugar) does not occur to any great extent in normal diets. Its concentration, even in mushrooms, is quite low (Birch 1963), although it does occur as the major blood sugar of many insects and in certain bacteria and other lower forms of life. Therefore, it is surprising that the enzyme α,α-trehalase, which is specific for α,α-trehalose, splitting it at the glycosidic linkage to form two molecules of glucose, is present in the human intestine. Evidence that man's ancestors were largely insectivorous has led Courtois (1970) to suggest that human intestinal trehalase may be a relic of some prehistoric digestive requirement. If some of the new fungal protein supplement foods come into extensive use, trehalose—which may be present in them in high concentration—could constitute a considerable portion of the diet; but this sugar does not appear to be present in any but trace quantities in human tissues.

Sacktor and Berger (1969) have shown that α,α-trehalase is abundant in human kidney tissue and that it is associated with glomerular reabsorption of glucose. This prompted them to assign an intrinsic and fundamental role to the disaccharide in the sugar transport process, but it is difficult to see how this can be so without *de novo* synthesis of trehalose, followed by its hydrolysis with the enzyme trehalase. Such a mechanism is not conceivable unless the presence of the sugar itself is convincingly demonstrated.

Glucose Syrup

Glucose syrup or liquid glucose is defined as an aqueous mixture of nutritive saccharides derived from starch. It is obtained from starch either by the action of acids or of enzymes; it is widely used in the food industry, about 150,000 tons being produced annually by the largest British manufacturer and considerably more by domestic manufacturers. Glucose syrup (Birch *et al.* 1970) is of interest in that

it represents the major source of rare food sugars in the diet, and although the normal range of types can be consumed without any unusual effects, glucose syrups may cause diarrhea in babies (MacDonald 1970). The rare sugar mixture of glucose syrup consists of the homologous series of maltodextrins which, consisting principally of $\alpha,1 \rightarrow 4$ glycosidically linked oligosaccharides, can be dealt with by the normal systems of amylases and other α-glycosidases present in the human digestive system. The less abundant, $\alpha,1 \rightarrow 6$ linkages can also normally be handled by the digestive enzymes; however, it is by no means certain that sugars containing $1 \rightarrow 2$, $1 \rightarrow 3$, or $1 \rightarrow 5$ linkages of the α-type or any linkages of the β-type can be digested normally by the human organism. Such rare food sugars can occur in certain types of glucose syrup by the "reversion" process in acid manufacture, by transglycosidation reactions, or by nonspecific reactions in enzyme manufacture (Birch and Kheiri 1970). Some glucose syrups can contain more than 10% of reversion products, and it is not known what effect these may have if consumed in large quantities.

Much has been written in recent years about the relationship between carbohydrate intake and lipid metabolism. Although some of the positions taken are unfounded, it has become increasingly clear that the metabolism of the different carbohydrates varies according to their molecular structure. This is of fundamental significance in the disorders of carbohydrate intolerance and malabsorption to be discussed in the following section.

Carbohydrate Intolerance and Malabsorption

During the 1960's the hitherto unknown diseases of carbohydrate intolerance and malabsorption were discovered and categorized. These can be classified under three headings (Dahlquist *et al.* 1968):

(1) Polysaccharide intolerance due to amylase dysfunction.
(2) Absence of the digestive disaccharidases of the small intestine.
(3) Malfunctioning of the absorptive process.

The first of these is caused mainly by the absence of pancreatic amylase, and the stools of such patients are often gray-colored, bulky, foul-smelling, and glistening. The second type of intolerance is frequently characterized by the lack of a specific disaccharidase, which causes the patient to be specifically intolerant of lactose, for example. On the other hand, two or more sugars may be able to cause this effect, and sucrose/isomaltose malabsorption may be encountered. In the third class of disorder the absorptive mucosa may be damaged or atrophied as a result of another primary disease, so that no carbohy-

drate can be absorbed by the alimentary system. In the specific case of glucose/galactose malabsorption, the transport process mediated by the sodium pump is not functioning properly, so that fructose may be found to be an alternative useful carbohydrate.

The most widespread and best understood type of carbohydrate intolerance is lactose intolerance. It is found that between 70 and 90% of all adult Thais, Africans, and Orientals suffer from the complaint. Hence it can be said to be the "normal state" for the majority of the world's adult population. Lactose intolerance is characterized by the inability of an individual to metabolize consumed milk and milk products due to the lactose content. For them, either milk or lactose, when taken orally, gives rise to cramping abdominal pains, bloating of the abdomen, flatulence, diarrhea, and borborygmia. As with all cases of carbohydrate intolerance, the only satisfactory treatment is to remove the offending carbohydrate from the diet. Lactose intolerance is due to the absence of the digestive enzyme lactase. This can be either congenital or acquired, but in the case of infants the disorder can be a major problem since the lactose content of human milk is somewhat higher than that of cows' milk. One solution is to add fungal lactase to the milk some time before ingestion, so that the lactose is completely hydrolyzed to glucose and galactose.

Diseases of carbohydrate digestion and absorption seem to affect the patient much more seriously in infancy and early childhood than in adult life. This is partly because the higher carbohydrate calorific value of the diet per unit of body weight in infancy produces a correspondingly greater demand on the pancreatic and mucosal enzymes and on the absorption mechanism of the mucosal surface. Since malabsorption is a common feature of the diseases, one method of diagnosis is to feed the suspected carbohydrate and examine the blood sugar curve. If it remains flat, the diagnosis is confirmed. Other diagnostic procedures are the examination of urinary and fecal sugars and mucosal biopsy.

METABOLISM OF CARBOHYDRATE

As can be seen from the equation at the beginning of this chapter carbohydrate has less than half the calorific value of fat and may vary considerably in its dietary proportions. The bulk of the calorific intake in certain parts of South America, for example, may be carbohydrate, whereas Eskimos may derive 80 to 90% of their calories from lipid. The average Western diet may contain 200 gm of carbohydrate, 70 gm of protein and 60 gm of fat, and the basal usage of Calories is about 1700 Calories per day. For an average man undertaking a considerable amount of physical exercise, the daily usage may be about

5000 Calories. Excess Calorie intake, whether in the form of carbohydrate, fat or protein, is converted into lipid and stored as such. On the other hand expenditure of Calories in excess of the amount consumed is met by mobilization of adipose tissue lipid with resulting loss in weight. Although excess blood sugar (following a meal) is converted into the reserve polysaccharide glycogen (in muscle and liver), the glycogen stores do not constitute more than a small part of the total body energy reserves, which are mainly lipid in nature, as shown in Table 5.7.

TABLE 5.7

FUEL RESERVES IN AN AVERAGE MAN

Chemical Nature	Source	Calories
Triglyceride	Adipose tissue	100,000
Glycogen	Liver	200
	Muscle	400
Glucose	Body fluids	40
Protein	Muscle	25,000

To convert the entire food intake into energy, 16 moles of oxygen are required daily and 13.7 moles of carbon dioxide are produced. Of the 16 moles of oxygen consumed, approximately one-fourth is utilized by nervous tissue, one-fifth by the liver, one-fifteenth by the kidneys and one-fifteenth by the heart. Approximately 120 gr ($^2/_3$ mole) of glucose are required each day by the brain. After intake the liver accommodates excess carbohydrate as glycogen, but the complete details of glycogen turnover rate in humans are not yet understood. In the rare diseases of excess glycogen storage, which afflict perhaps 1 in 40,000 of the population, certain amylases and associated enzymes are missing from the liver tissue, so that glycogen builds up in this organ to intolerable levels, with consequent dysfunction. Control of dietary carbohydrate is about the only successful therapeutic measure. Many patients suffering from these diseases die in infancy because of lack of speedy diagnosis.

The two main pathways by which carbohydrate is converted to energy are the Embden-Meyerhof-Parnas (EMP) scheme of glycolysis and the Krebs tricarboxylic acid cycle. The former operates under anaerobic conditions and allows the stepwise conversion of glucose or glycogen into pyruvate and then lactate, which may be decarboxylated to acetaldehyde and reduced to ethanol. In the Krebs tricarboxylic acid cycle, the pyruvic acid produced via the EMP pathway (or otherwise obtained via lipid metabolic pathways to fatty acids) is con-

verted to citric acid, *cis*-aconitic acid, isocitric acid, oxalosuccinic acid, α-ketoglutaric acid, and carbon dioxide. The α-ketoglutaric acid so formed can then be converted to succinic acid and thence to fumaric and malic acids. The latter, through the intermediation of coenzyme A, is reconverted to citric acid, thus completing the cycle. The enzymes of the citric acid cycle are closely associated with those of electron-transfer mechanisms of the respiratory chain, and with those of the phosphorylation of adenosine diphosphate (ADP). The net result of the EMP and the Krebs tricarboxylic acid cycles is that a steady state of oxidation of carbohydrate can be achieved, with consequent synthesis of relatively large amounts of adenosine triphosphate (ATP). Finally, it is from the ATP itself that the energy needed to drive the resynthesis of essential cellular components (proteins, polysaccharides, etc.) and to perform essential biological functions (muscle contraction etc.) is directly derived. Both the above cycles are freely reversible, so that excess fatty acid may be reconverted to carbohydrate (glucose and glycogen). This is known as glucogenesis. Since it is clear from the foregoing that the total carbohydrate of the body is barely sufficient (Table 5.7) to meet the demands of nervous tissue, glucogenesis is probably a highly significant metabolic process. Because carbohydrate provides the greater part by weight of an average Western diet and yet constitutes such a small proportion of the total tissue weight, it is also clear that carbohydrate-lipid interactions are of great importance in mammalian nutrition. Furthermore, it is now quite evident that the molecular structure of the various dietary sugars will govern their metabolic fate. Previous assumptions that all dietary carbohydrates were freely converted to glucose and thus similar in their metabolic effects are now known to be erroneous.

Carbohydrate-lipid Interactions

The three ways in which dietary carbohydrate influences lipid production are usually measured as total body fat, liver lipid, and blood lipid. The latter two may be further divided into phospholipid, triglyceride, free fatty acid (FFA), and cholesterol. There is now a considerable body of evidence (MacDonald 1971) that the amount and type of dietary carbohydrate will govern the tissue levels of these lipids provided that particular types of lipid (e.g., cholesterol) are already present in the diet (Allen and Leahy 1966; Al-Nagdy *et al.* 1970).

So much interest has been aroused in recent years about the effect of diet on the incidence of coronary heart disease that carbohydrate (particularly sucrose, glucose, and starch) and fat have been closely

scrutinized. The dietary intake may or may not control the build-up of atheroma on the arterial walls that is involved in the disease state. Men, and women over the age of menopause, are particularly prone to coronary heart disease, so that sex hormones are decidedly implicated in protection against the disease and the steroid structure of cholesterol may thus be a clue to the effect.

Since changes in the tissue cholesterol levels that follow particular types of carbohydrate intake depend on the amount of cholesterol itself present in the diet, it is difficult to assess the part played by dietary carbohydrate on the level of this lipid. In rats and rabbits replacement of dietary starch with sucrose results in an increase of serum cholesterol level (MacDonald 1971) so long as cholesterol is present in the diet. The effects of dietary sucrose on cholesterol levels in man are not clear. Serum phospholipid also does not appear to change consistently with increase in dietary sucrose.

It seems that dietary carbohydrate does not have any striking effect on serum triglyceride levels within a few hours of ingestion, except a slight *reduction* in level probably due to the action of insulin. However, after 10 to 12 hr marked changes in the fasting level become obvious. Most work in this field so far has been done on the rat, and the effects reported depend on the age, strain and sex of the rats used. Also, in the rat the absorption of fructose is quite different from that in man, in that after hydrolysis of sucrose by intestinal invertase the fructose liberated diffuses back into the intestinal lumen and is absorbed lower down in the gut. In male baboons dietary sucrose causes an increase in fasting serum triglyceride level during the first 3 weeks, followed by a gradual drop, so that after 17 weeks the level is the same as in controls. No such changes occur in female baboons. Estrogen may enhance the removal of triglyceride from the serum of female animals (Colthart and MacDonald 1971), but it is not clear whether the effects observed with male baboons are due to the entire sucrose molecule or only to the fructose moiety.

In humans the effects are more distinct. Diets containing fructose raise the fasting serum triglycerides of men, but the reverse effect seems to occur in young women (Antar and Ohlson 1965; MacDonald 1966). Glucose syrups appear to behave metabolically in a manner that resembles glucose and starch rather than sucrose (MacDonald 1970), so that male mammals may also show lower fasting levels as a result of ingesting this complex mixture of sugars. However, as already indicated in this chapter, glucose syrup can be considered as a highly complex mixture of rare food sugars, and the net biological effect of all its individual components will take years to elucidate. One recent observation (Birch *et al.* 1973; Chalvardjian and Stephens 1970) is that the high molecular weight fraction of glucose syrup is

able to cause swollen caeca in rats. This occurs within a few days of ingestion and caeca become about twice normal size; as far as can be seen, this causes no overt ill-effects. This phenomenon may be due to selective attack by gut microorganisms on particular high molecular weight polymers in the mixture.

Dietary Fiber

Crude fiber is an insoluble organic component of the diet consisting mainly of cellulose, hemicellulose, plant gums and mucilages, and a little lignin. Although recommended as a well-known form of "roughage" in the diet, it is only in recent years that it has begun to attract serious scientific consideration (Painter 1970). In facilitating the passage of the food mass through the intestine after ingestion, fiber prevents the concentration of localized pressures and hence lowers the average gut residence time, which may be defined as the interval between ingestion of food and excretion of the residue. Gut residence time averages about 72 hr for a normal Westerner, whereas for Africans, whose diet is much more fibrous, the residence time is only 48 hr on the average. Obviously, the shorter residence time lowers the possibility of attack by gut microorganisms and hence reduces flatulence. Furthermore, fiber appears to affect bile secretion, so that serum cholesterol levels may be lowered on a diet containing sufficient fiber. Africans living on indigenous diets are known to have serum cholesterol levels far lower than the average westerner. Also the condition of diverticulitis of the colon is almost unknown in African communities.

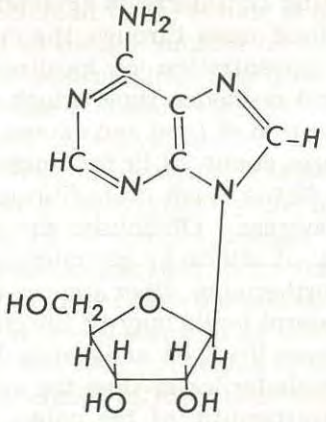
Athletic Nutrition

For a given available quantity of oxygen, carbohydrates will supply more energy (Calories) than either fat or protein. Thus athletes prepare for major events by consuming high-protein diets for muscle building, but switch to a high carbohydrate diet a short while before the event. If energy is required quickly and in large amounts for an athletic event, it is important to supply it by means of selected carbohydrates that pass rapidly from the stomach (counteracting as far as possible the delay in pyloric opening that may accompany nervousness) and are absorbed rapidly in the small intestine. It is known that certain glucose syrups are able to elevate blood glucose levels at an hitherto unexplained rate, and athletic drinks based on these are supplied to British Olympic athletes. On the other hand, work done as far back as 1955 by the Sports College of Canada indicated that honey is the most valuable carbohydrate food for athletes because of its digestibility, acidity, general tolerance and caloric content, as well as taste appeal. Honey is alleged to possess several additional nutri-

tional advantages, including an anti-infection agent (inhibine) and benefit to heart patients. Its composition has never been fully elucidated, but maltose and many other rare food sugars are present. In view of the reported biological effects these obviously deserve fundamental study.

NUCLEOSIDES, NUCLEOTIDES, AND NUCLEIC ACIDS

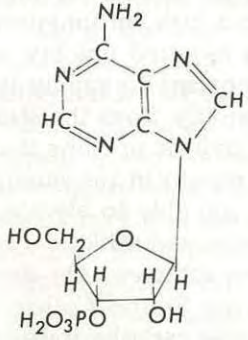
When a sugar is condensed with a purine or pyrimidine base to give a product of the glycosylamine type it is referred to as a nucleoside. An example is the well-known substance adenosine:



ADENOSINE

(9-β-D-RIBOFURANOSIDOADENINE)

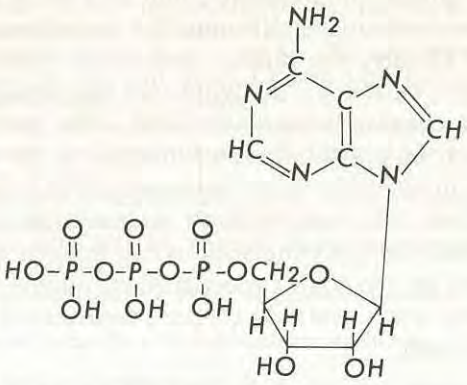
Nucleosides may be esterified with phosphoric acid to give nucleotides. An example of the latter is adenosine monophosphate (adenylic acid):



(YEAST) ADENYLIC ACID

(ADENOSINE-3′-PHOSPHATE)

However, two or three phosphate groups may be esterified in this way in nucleotides and in the example given this would result in the formation of adenosine diphosphate (ADP) or adenosine triphosphate (ATP).



ADENOSINE TRIPHOSPHATE (ATP)

Chains of nucleotides may be formed by joining the pentoses of nucleotides via their phosphate esters, usually at position 3′ and 5′ of the pentose to give a product known as a polynucleotide or nucleic acid.

In the nucleic acids at least 4 different bases may be present in the chain and their sequential arrangement may vary. The pentose of the nucleic acid is either ribose or 2-deoxyribose. The former gives rise to ribonucleic acid (RNA), whereas the latter gives deoxyribonucleic acid (DNA). It was originally thought that DNA was a product of animal tissues whereas the RNA came from plant sources. Although both types of nucleic acid occur in plant and animal tissues, DNA is exclusively a nuclear material, and RNA occurs chiefly in the cytoplasm. The nuclei of tissue cells from a single species contain relatively constant amounts of DNA per nucleus; thus a determination of the quantity of DNA in a tissue permits an estimate to be made of the number of cells in that tissue. The nucleic acids control the genetic mechanism of cells. Evidently they form a template on which new protein is synthesized, and this is determined by the order in which the purine and pyrimidine bases occur along the polynucleotide chain. The entire arrangement is helical in structure, and the molecular weight of DNA may be about 6 million.

The nucleotides are involved in many important biological reactions. For example, the electron carriers, nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP), are involved in the EMP glycolytic pathway and the Krebs tricarboxylic

acid cycle previously mentioned. The nucleotide, uridine diphosphoglucose (UDP), has a fundamental role in the biosynthesis of a number of important oligosaccharides. α,α-Trehalose (mushroom sugar), for example, is formed first as its phosphorylated intermediate from UDP and glucose 6-phosphate, and sucrose is formed in sugar cane by a similar mechanism. Vitamin B_{12} (cobalamin) has a nucleotide structure. Finally, the almost ubiquitous functions of ATP as the direct energy "currency" of living cells has already been referred to. This energy appears to be made available as the result of hydrolysis of one or more of the phosphate ester linkages in the molecule.

Many of the nucleotides have flavor-enhancing properties, which have brought them into use by food technologists in recent years. On the other hand, the polynucleotides can present nutritional problems if consumed in too high concentration. Some of the new bacterial cell protein foods can lead to the condition of hyperuricaemia (gout) for this reason.

BIBLIOGRAPHY

ACTON, E. M., CHRISTENSEN, J. E., STONE, G., and GOODMAN, L. 1968. Synthesis and taste properties of an N-(Glucose)-saccharin compound. Experentia *24*, 998-999.

ALLEN, R. J. L., and LEAHY, J. S. 1966. Some effects of dietary dextrose, fructose, liquid glucose and sucrose in the adult male rat. Brit. J. Nutr. *20*, 339.

AL-NAGDY, S., MILLER, D. S., and YUDKIN, J. 1970. Changes in body composition and metabolism induced by sucrose in the rat. Nutr. Metabol. *12*, 193-219.

ALPERS, D. H., and SOLIN, M. 1970. The characterization of rat intestinal amylase. Gastroenterol. *58*, 833-842.

ANTAR, M. A., and OHLSON, M. A. 1965. Effect of simple and complex carbohydrates upon total lipids, non-phospholipids, and different fractions of phospholipids of serum in young men and women. J. Nutr. *5*, 329-337.

BARKER, S. A., BRIMACOMBE, J. S., FOSTER, A. B., WHIFFEN, D. H., and ZWEIFEL, G. 1959. Aspects of stereochemistry. II. Intramolecular hydrogen bonding in some monohydroxy derivatives of tetrahydrofuran, tetrahydropyran and 1:3 dioxane. Tetrahedron 7, 10-18.

BARNETT, J. E. G., RALPH, A., and MUNDAY, K. A. 1970. Structural requirements for intestinal transport. The nature of the carrier-sugar binding at C-2 and the ring oxygen of the sugar. Biochem. J. *118*, 843-850.

BEIDLER, L. M. 1971. The physical basis of the sweet response. *In* Sweetness and Sweeteners, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.

BIESTER, A., WOOD, M. W., and WAHLIN, C. S. 1925. Relative sweetness of sugars. Am. J. Physiol. *73*, 387-396.

BIRCH, G. G. 1963. Trehaloses. Advances in Carbohydrate Chem. *18*, 201-225.

BIRCH, G. G. 1969. Rare food sugars. Food World, *4*, 5-7.

BIRCH, G. G. 1973. Sub-threshold enhancement of sweetness. Unpublished results.

BIRCH, G. G., COWELL, N. D., and EYTON, D. 1970. A quantitative investigation of Shallenberger's sweetness hypothesis. J. Food Technol. *5*, 277-280.

BIRCH, G. G., COWELL, N. D., and YOUNG, R. 1973. The structural basis of

sweetness and bitterness in foods and their interactions. J. Sci. Food Agr. *23*, 1207-1212.

BIRCH, G. G., ETHERIDGE, I. J., and GREEN, L. F. 1973. Short term effects of feeding glucose syrup fractions in rats. Brit. J. Nutr. *29*, 87-93.

BIRCH, G. G., GREEN, L. F., and COULSON, C. B. 1970. Glucose Syrups and Related Carbohydrates. Elsevier Publishing Co., Amsterdam.

BIRCH, G. G., GREEN, L. F., and COULSON, C. B. 1971. Sweetness and Sweeteners. Applied Science Publishers, London.

BIRCH, G. G., and KHEIRI, M. S. A. 1970. The carbohydrates of glucose syrup. *In* Glucose Syrups and Related Carbohydrates, G. G. Birch *et al.* (Editors). Elsevier Publishing Co., London.

BIRCH, G. G., and LEE, C. K. 1971. The chemical basis of sweetness in model sugars. *In* Sweetness and Sweeteners, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.

BIRCH, G. G., LEE, C. K., and ROLFE, E. J. 1971. Organoleptic effect in sugar structures. J. Sci. Food Agr. *21*, 650-653.

BIRCH, G. G., and LINDLEY, M. 1973. Structural analogies in saporific sugars. Unpublished results.

BIRCH, G. G., and RICHARDSON, A. C. R. 1968. Chemical modification of trehalose. Part I. Selective sulphorylation of trehalose, and determination of the conformation of hexa-*O*-acetyl-6,6′-dideoxy-trehalose. Carbohyd. Res. *8*, 411-415.

BIRCH, G. G., and SHALLENBERGER, R. S. 1973. Configuration and conformation of food sugars. *In* Molecular Structure and Function of Food Carbohydrate, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.

CHALVARDJIAN, A., and STEPHENS, S. 1970. Lipotropic effect of dextrin versus sucrose in choline-deficient rats. J. Nutr. *100*, 397-403.

COHN, G. 1914. The Organic Taste Compounds. Siemenroth, Berlin.

COLTHART, T. M., and MACDONALD, I. 1971. Effect of sex hormones on fasting serum triglycerides in baboons given high-sucrose diets. Brit. J. Nutr. *25*, 323.

COURTOIS, E. 1970. Evolution of the knowledge in the field of trehalose and trehalase. Fifth International Symposium on Carbohydrates, Paris.

CRANE, R. K. 1968. The digestion and absorption of carbohydrates. *In* Carbohydrate Metabolism and its Disorders, Vol. I, F. Dickens *et al.* (Editors). Academic Press, New York.

DAHLQUIST, A., LINDQUIST, B., and MEEUWISSE, G. 1968. Disturbances of the digestion and absorption of carbohydrates. *In* Carbohydrate Metabolism and its Disorders, Vol. II, F. Dickens *et al.* (Editors). Academic Press, New York.

DASTOLI, F. R., and PRICE, S. 1966. Sweet-sensitive protein isolated from bovine taste buds; isolation and assay. Science *154*, 905-907.

DEUTSCH, E. W., and HANSCH, C. 1966. Dependence of relative sweetness on hydrophobic bonding. Nature *211*, 75.

EVANS, D. R. 1963. Chemical structure and stimulation by carbohydrates. *In* Olfaction and Taste, Y. Zotterman (Editor). International Symposium Series, Vol I. Oxford Univ. Press, London.

GOLDFINE, I. D., ABRAIRA, C., GRUENWALD, D., and GOLDSTEIN, M. S. 1970. Plasma insulin levels during imaginary food ingestion under hypnosis. Proc. Soc. Exp. Biol. Med. *133*, 274-276.

HENKIN, R. I., and SHALLENBERGER, R. S. 1970. Aglycogeusia: the inability to recognize sweetness and its possible molecular basis. Nature *227*, 965-966.

KARE, M. 1969. Some functions of the sense of taste. J. Agr. Food Chem. *17*, 677-680.

KIER, L. B. 1972. A molecular theory of sweet taste. J. Pharm. Sci. *61*, 1394-1397.

KODAMA, S. 1920. Taste. J. Tokyo Chem. Soc. *41*, 495-534.
MACDONALD, I. 1966. Lipid response of postmenopausal women to dietary carbohydrates. Am. J. Clin. Nutr. *18*, 86-90.
MACDONALD, I. 1970. Some effects of consuming glucose syrups. *In* Glucose Syrups and Related Carbohydrates, G. G. Birch *et al.* (Editors). Elsevier Publishing Co., Amsterdam.
MACDONALD, I. 1971. Sugar and blood lipids. *In* Sugar, J. Yudkin *et al.* (Editors). Butterworths, London.
OERTLY, E., and MYERS, R. G. 1919. A new theory relating constitution to taste. J. Am. Chem. Soc. *41*, 855-867.
PAINTER, N. S. 1970. Pressures on the colon related to diverticular disease. Proc. R. Soc. Med. *63*, 144-147.
PAULING, L. 1960. The Nature of the Chemical Bond. Cornell University Press, Ithaca, New York.
RICHTER, C. P., and DUKE, J. R. 1970. Cataracts produced in rats by yoghurt. Science *168*, 1372-1374.
SACKTOR, B., and BERGER, S. J. 1969. Formation of trehalose in the renal cortex. Biochem. Biophys. Res. Commun. *35*, 796-800.
SCHUTZ, H. G., and PILGRIM, F. J. 1957. Differential sensitivity in gustation. J. Exptl. Psychol. *54*, 41-48.
SHALLENBERGER, R. S. 1963. Hydrogen bonding and the varying sweetness of the sugars. J. Food Sci. *28*, 584-589.
SHALLENBERGER, R. S. 1964. Sweetening agents in foods. Agr. Sci. Rev. *2*, 11-20.
SHALLENBERGER, R. S. 1964. Why do sugars taste sweet? New Scientist *407*, 569-570.
SHALLENBERGER, R. S. 1971. The theory of sweetness. *In* Sweetness and Sweeteners. G. G. Birch *et al.* (Editors). Applied Science Publishers Ltd., London.
SHALLENBERGER, R. S. 1973. Molecular structure of compounds and the chemical nature of receptor sites for the chemical senses. *In* Pharmacology and the Future of Man. Proc. 5th Int. Congr. Pharmacology, San Francisco, 1972, Vol. 5. Karger, Basel.
SHALLENBERGER, R. S., and ACREE, T. E. 1967. Molecular theory of sweet taste. Nature *216*, 480-482.
SHALLENBERGER, R. S., and ACREE, T. E. 1969. Molecular structure and sweet taste. J. Agr. Food Chem. *17*, 701-703.
SHALLENBERGER, R. S., and ACREE, T. E. 1971. Chemical structure of compounds and their sweet and bitter taste. *In* Handbook of Sensory Physiology, IV. Chemical Senses, 2. Taste, L. M. Beidler (Editor). Springer-Verlag. Berlin.
SHALLENBERGER, R. S., ACREE, T. E., and LEE, C. Y. 1969. Sweet taste of D- and L-sugars and amino acids and the steric nature of their receptor site. Nature *221*, 555-556.
SHAPIRA, J. 1967. Approaches to the chemical synthesis of food. NASA-TM-X-59839. Thesis of Food N68-27463.
SPENCER, H. W. 1971. Taste panels and the measurement of sweetness. *In* Sweetness and Sweeteners, G. G. Birch, *et al.* (Editors). Applied Science Publishers, London.
TSUZUKI, Y. 1948. Sweet taste and chemical constitution. Chemistry and Chem. Ind. (Japan) *1*, 32-40.
VAN HEYNINGEN, R. 1969. Xylose cataract: A comparison between the weanling and the older rat. Exptl. Eye Res. *8*, 379-385.
WARFIELD, R. B. 1954. Taste and molecular structure. Abstr. Papers. Am. Chem. Soc. 126th meeting Division of Agr. and Food Chem. p. 15a.
WELLS, D. A. 1858. Principles and Applications of Chemistry. Ivison and Phinney, New York.

Role of Sugars in Food and Medicine

Until about 25 years ago the only sugars likely to occur in any significant amounts were the three common types, sucrose (cane or beet sugar), D-glucose and D-fructose. The latter two sugars can be generated in equimolar concentration by acid or enzymic hydrolysis of sucrose. These preparations are known as "invert" sugar. The term invert signifies that when sucrose is hydrolyzed the specific rotation changes from a positive value (+66.5°) to a negative value (ca. -20°). This is because fructose is more strongly levorotatory than glucose is dextrorotatory:

$$+66.5^\circ \longrightarrow \frac{(-92^\circ) + (+52^\circ)}{2} = -20^\circ$$

This usual meaning of the term invert (or inversion) in sugar chemistry is mentioned here to distinguish it from the geometrical operation of "inverting" a sugar conformation.

Glucose syrups with tailored properties are used as food ingredients and are intentionally added. Besides their use for their well-known caloric and sweetening properties, they are also used as thickening and bodying agents and for preservative value. Newer roles in the fields of flavor, dietary control, and precise clinical design of foodstuffs are in the course of discovery. Fundamental chemical understanding of afflictions such as dental caries and atherosclerosis is beginning to indicate dietary control methods based on the chemistry of food sugars.

Together with greater chemical knowledge of the food sugars has come an advance in analytical techniques for assessing and controlling the concentrations of sugars in foods, and for monitoring the changes that take place during processing and storage. The same techniques may be applied to attempts to understand the metabolic changes that take place on ingestion of carbohydrates and the range of sugar types excreted by the human organism as end products of these changes. Finally, although a number of simple sugars or their derivatives have been used therapeutically, it must be admitted that this application has so far met with only limited success except in those idiosyncratic disorders that can be treated by a complete change in dietary carbohydrate.

FOOD

Types of Food Sugar

The most abundant food sugar is, of course, sucrose (common sugar, cane sugar, beet sugar, or saccharose) and it is also the most abundant and purest food chemical in the diet. Commercial white sugar contains between 99.9% and 99.99% sucrose and is free of microorganisms. However, traces of impurities may govern its catalytic hydrolysis or thermal degradation under different conditions of processing. Hence caramel and other colored degradation products, which may be a problem in the manufacture of some foods, can be traced to the type and concentration of minerals in commercial sugar used as a raw material. The various raw and brown types of sugar on the market may have as little as 95% sucrose and much higher mineral content; iceing sugar is simply finely powdered sucrose containing some suitable anticaking agent such as starch (about 5%) or an inert inorganic type such as magnesium stearate (about 1.5%). Anticaking agents prevent the absorption of water by the finely divided sugar and have been approved for food use by the Codex Alimentarius International Sugars Commission.

Sucrose is by far the most abundant sweetening agent used in foods throughout the world, about ten million tons being produced annually in the United States from cane and beet sugar. However, the cost of sucrose has allowed the incursion of alternative sweeteners such as the commercial starch hydrolyzates known as glucose syrups or "liquid glucose" and dextrose, of which about 600,000 tons are produced annually in the world (Hodge *et al.* 1972). Glucose syrup is made from starch (chiefly cornstarch) either by the action of acids or enzymes (gluco-amylase) and consists of D-glucose, maltose, and a series of malto-dextrins. In the United States these substances are termed corn syrups to indicate their source, but the preferred Codex Alimentarius name for them is "glucose syrup," and they are defined as aqueous mixtures of nutritive saccharides derived from starch.

Glucose syrups are characterized commercially by their dextrose equivalents (D.E.), i.e., their reducing power (to Fehling's solution) calculated as dextrose and expressed on a dry weight basis. Thus starch that has undergone a high degree of conversion will obviously consist largely of D-glucose and will therefore have a high D.E., whereas low degrees of conversion give glucose syrups of low D.E. Since sugars above the molecular weight of trisaccharide in these series have no sweetness (Chapter 5) it is clear that only high or intermediate degrees of conversion are suitable for producing sweetening power, whereas low-D.E. glucose syrups have other uses in foods (e.g., bodying agents in soft-drink manufacture).

Most commercial glucose syrups have a D.E. in the range 20 to 70. If starch could be solubilized without degradation prior to acid hydrolysis, the distribution of saccharides at any stage of the conversion process should be calculable by assuming random attack by protons (Painter 1963). However, since the initial stages of hydrolysis take place while the starch is still present in slurry form, the sugar distribution bears no resemblance to that calculated (Birch 1968). These sugar "spectra" in glucose syrups can only be obtained by laborious and time-consuming analyses, and it is very difficult to determine sugars with degrees of polymerization greater than 8 (i.e., with a molecular weight greater than 1500).

Fructose is the sweetest known sugar, and recently some commercial moves have been made to introduce it as a sweetening agent. The sugar is usually obtained from invert sugar. However, its present high cost will probably preclude its adoption on any significant scale in the food industry in the near future. Attempts have been made to produce "fructose syrups" from glucose syrups by treating ordinary glucose syrups with an enzyme called "isomerase." A number of fructose syrups containing at least 45% fructose are now available and are claimed to have sweetening powers approaching that of sucrose. Here again they are more costly than the common glucose syrups and their complete carbohydrate spectra are not known. Yet another form of syrup in use by food manufacturers is "sucrose syrup" which consists of an aqueous sucrose solution with or without invert sugar and/or glucose syrup. Because of the extreme ease of crystallization of sucrose in these syrups they cannot normally be stored at strengths greater than 67% w/v, which is considerably lower than that of the glucose syrups (ca. 80% w/v). Nevertheless sucrose syrups are probably more widely used than glucose syrups by food manufacturers in the United States. Mixtures of sucrose syrup and glucose syrup are available for commercial use.

Apart from the rare food sugar mixture present in glucose syrup itself as a series of maltodextrins, maltose may be used as a food-grade disaccharide in confectionery, or may be present as the major component of enzymically prepared (high-maltose) glucose syrup. Lactose (Birch 1972) is another food sugar that occurs in fresh and concentrated milks and milk products; a new form of lactose consumption has emerged with the dramatic growth in the yoghurt market in the last decade.

Less common food sugars or sugar derivatives now being suggested for particular sweetening or flavoring purposes are sorbitol (mainly in diabetic foods), xylose and xylitol, ribose, arabinose, mannose and galactose. Various combinations of these monosaccharides constitute

some of the minor carbohydrate fractions of sugar beet, cereals and beans, whereas the new fungal protein foods may contain the disaccharide α,α-trehalose. The diabetogenic monosaccharide mannoheptulose is found in the avocado pear.

Honey, the oldest sweetener known to man (prehistoric cave paintings in Spain depict its collection from nests of wild bees 15,000 years ago) consists of invert sugar with smaller amounts of other types, principally maltose, not sucrose.

Taste of Food Sugars

The structural basis of sweetness has been discussed in Chapter 5, but the particular sweetness and flavor characteristics of the mixture of sugars that may be selected for a particular food purpose depend on the commercial feasibility of using such a mixture. Thus, although the sugars have a purer sweetness than the artificial sweetening agents, which may possess bitterness or other flavors, the latter are selected for their lower sweetness cost and their greater intrinsic sweetness (Green 1971), as shown in Table 6.1.

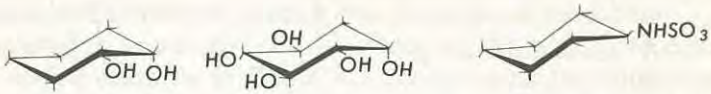
TABLE 6.1

RELATIVE SWEETNESS OF VARIOUS SUBSTANCES

Substance	Relative Sweetness, Weight Basis
Sucrose	1
Cyclohexylsulfamate (sodium cyclamate)	30
L-Aspartyl-L-phenylalanine methyl ester	100
2, 3-Dihydro-3-oxobenzisosulfonazol (saccharin)	350
Neohesperidin dihydrochalcone	1000
Perillaldehyde *anti*oxime (perillartine)	2000
1-*n*-Propoxy-2-amino-4-nitrobenzene (P-4000)	4000

Until 1970 the only artificial sweeteners allowed in foods in the United States and United Kingdom were saccharin and cyclamate; now only saccharin remains on the permitted list, although it no longer enjoys the Federal Drug Administration's GRAS (generally recognized as safe) status. The doubts about these two artificial sweeteners, both of which contain nitrogen, have stimulated the search for a new artificial sweetener that more nearly approximates the chemical structure of the carbohydrates (nitrogen-free) while possessing the basic structural requirements of an intensely sweet molecule. Along these lines, consideration of the structures of three 6-membered ring molecules that differ markedly in their tastes, as

shown below, leads to the speculative possibility that the basic structural unit for intense sweetness may be an intact cyclohexane ring with the polar AH, B system outside:



TASTELESS SWEET INTENSELY SWEET

Sugars vary in their taste because of their intrinsic textural properties which cause differences in mouth feel and bodying effects in foods. Sucrose, for example, has a strong bodying effect; while some people may find this more cloying on the palate than D-glucose, the latter is said to be nauseating at a certain dietary level for most people and is therefore not preferred. When sucrose is mixed with artificial sweeteners in soft-drink formulations, the resulting loss in viscosity may have to be compensated by the addition of a suitable thickening agent such as an edible gum or synthetic emulsifiers and stabilizers. Sweetness varies with concentration; thus, while the accepted sweetness of cyclamate is 30 times greater than that of sucrose, it may be as high as 60 to 120 times that of sucrose in citrus drink formulations (Brook 1970). On the other hand, sweetness may be depressed by either acidity (Green 1971) or bitterness (Birch 1972), so that the net effect of all the components encountered in a food formulation may be complicated to assess.

Relative Sweetness of Sugars

Psychophysical Laws.—In judging either the quality or the magnitude of a sensory attribute, and relating it to a physical stimulus, such as the sweetness of a sugar at a given concentration, one immediately encounters the "psychophysical laws," and also the opinion (expressed by those who do not seem to have any need to quantify sensory phenomena) that sensory experience cannot be reduced to numbers. Nevertheless, the quantification of psychological phenomena seems to be well on the way to accepted scientific and mathematical expression.

Briefly, the psychophysical law states that the strength of a stimulus must be increased in geometric proportion in order to elicit an arithmetic increase in sensation (Fig. 6.1).

The mathematical relation for this phenomenon is:

$$S = kI^n$$

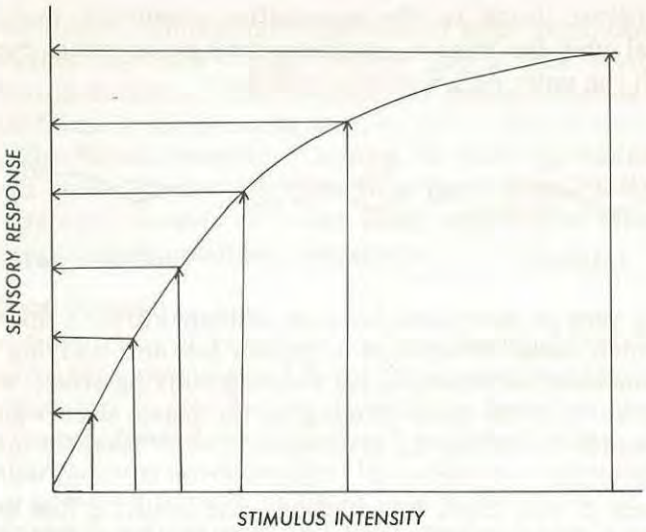


FIG. 6.1. RELATION BETWEEN ARITHMETIC INCREASE IN SENSORY RESPONSE AND GEOMETRIC INCREASE IN STIMULUS INTENSITY

where S is the *sensation*, and I is the *intensity* of the stimulus (e.g., increasing sugar concentration). As a log-log function, the relation is:

$$\log S = n \log I + \log k$$

where n is the slope and k is the intercept. For the sugars, n is usually 1.3, with a range, however, of 0.62 to 2.2 (Stone and Oliver 1969; Moskowitz 1970A). For this reason results of sweetness judgements for the sugars are sometimes found plotted on log-log coordinates, but over a relatively narrow range of sugar concentrations such plots do not always seem to be necessary.

Threshold Concentrations for the Sugars.—In spite of the difficulty of determining a threshold (limen) concentration for a sugar and its significance or meaning, such values have application. The "recognition" threshold concentrations for the sugars (Stone and Oliver 1969) are sucrose, 0.01*M* (0.3%); glucose, 0.037*M* (0.7%); and fructose 0.013*M* (0.2%). The "detection threshold" or the ability to detect the presence of a sugar in solution, as opposed to the ability to recognize the attribute of sweetness, is characterized by lower values.

Iso-sweet Sugar Concentrations.—Often, in using sugars as sweetening agents in foods, it is necessary to alter the sugars used for reasons

of supply, cost, etc., and the problem posed usually leads to the question "What amount of sugar y is needed to duplicate the sweetness of sugar x?" There is no straightforward answer to this seemingly simple question because the sweetness attributes of the sugars are not the same, and sweetness is not a one-dimensional sensation.

Single-value scores for the sweetness of the sugars are commonly used, as for example in Table 5.3, but it must be emphasized that the various sweetness properties included in these "integrated" values encompass impact or recognition time (t), the taste intensity (i), duration of the taste sensation (d), and other factors, including interaction with the other basic tastes (salty, sour, bitter), and tactile (k) sensations, or

$$\text{Sweetness } (S) = S(t, i, d, k, \text{etc.})$$

It is quite possible that a sugar with rapid impact ability but low taste intensity may give about the same sweetness score as one with slow impact time but greater taste intensity, particularly when a judgement is made at a specific time after tasting the solutions. In Fig. 6.2 sugar A has twice the impact rate but only one-half the taste intensity of sugar B, yet both would probably be judged to be about equally sweet using conventional organoleptic tasting procedures, since the integrated areas under the curves at time t are about the

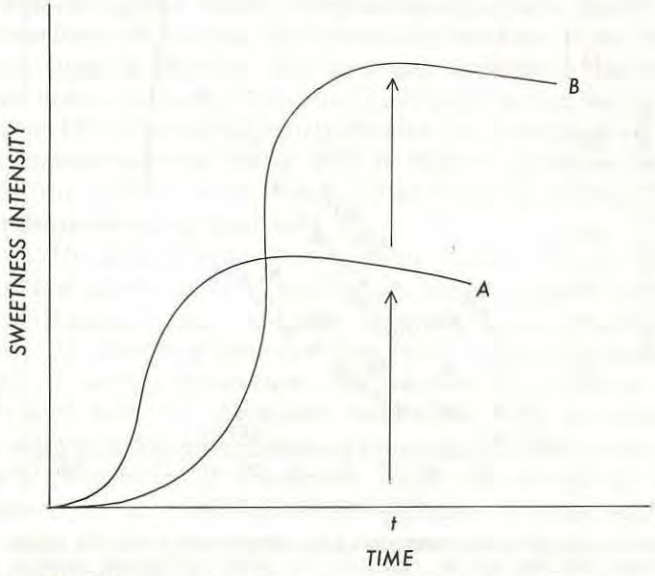


FIG. 6.2. SWEETNESS IMPACT TIME, INTENSITY CHARACTERISTICS OF HYPOTHETICAL SUGARS A AND B

same. Nevertheless, these different attributes are an important property in foods. It has been suggested (Shallenberger and Acree 1971) that the "impact" attribute of a sweet-tasting compound is related to degree of hydrophobic character, and its sweet taste intensity is related to the likelihood that its AH,B unit will interact with the taste bud receptor site.

The variable sweetness of the different sugars and reasons for it have even more striking analogies for the other basic tastes. Sodium chloride is the standard for salty taste, and its taste cannot be duplicated by any other compound. A person on a low-sodium diet who must season his food with the closely related salt, potassium chloride, will attest to this fact. Hydrochloric acid is probably the closest approach that can be made to a "true" sour taste, and other acids (e.g., acetic acid and citric acid) have additional tastes.

Approximate "iso-sweet" concentrations of various sugars with those of sucrose at various concentrations are shown in Fig. 6.3. The data shown are those of Dahlberg and Penzek (1941). According to these authors the sweetness of glucose at high solution concentrations (> 40%) is indistinguishable from that of sucrose. Other investigators (Yamaguchi *et al.* 1970; Cameron 1947) report data similar to that shown in Fig. 6.3. Stone and Oliver (1969) also report on the relative sweetness of sugar mixtures.

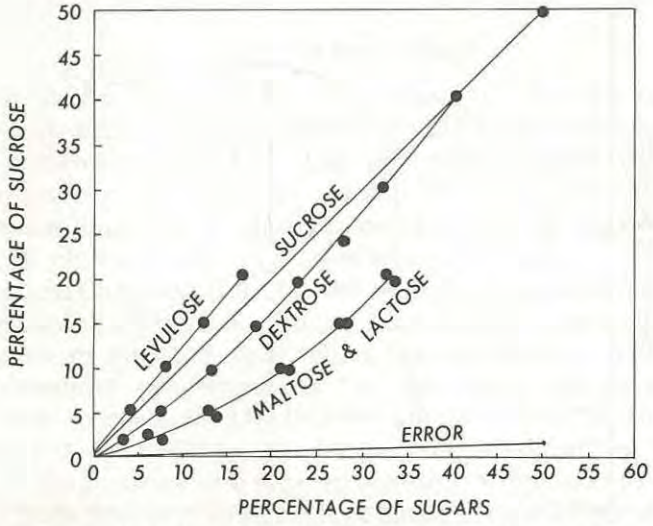


FIG. 6.3. CONCENTRATIONS OF DIFFERENT SUGARS, ON A DRY SOLIDS BASIS, NEEDED TO GIVE THE SAME SWEETNESS AS SUCROSE SOLUTIONS OF VARIOUS CONCENTRATIONS

Supplemental Taste Action of Sugars.—Without question, sugars have a supplemental action upon each others' intrinsic sweetness. This is often described as "synergism," which is an interplay of the various factors just mentioned (impact time, etc.) and results in a higher apparent sweetness. To paraphrase the practical experience of Dahlberg and Penzek (1941), in a mixture of sugars the sweetness of one is quickly perceived, promptly reaches a maximum intensity, and then decreases. The sweetness of the second sugar stimulates the sensory organs more slowly, and reaches maximum intensity later. Hence, the one sugar might be expected to supplement the other. Other investigators (Cameron 1947; Herrmann 1973) believe, however, that the reported synergism is simply an additive effect. For example, the addition of a given amount of fructose to glucose solutions has the same effect as the addition of a glucose concentration of the same sweetness, in spite of the fact that fructose and glucose are quite different sugars.

Honey, which is a unique blend of many sugars, possibly presents an example that is worthy of study with a view to technological utilization of designed mixtures. Honey is considerably sweeter than high-D.E. glucose syrups, and it therefore appears that a complete understanding of synergism as it exists in the mixture of low molecular weight sugars that occur in honey should precede attempts to control the sweetness of foods by chemical modification.

Sweetness of Invert Sugar.—Depending upon the values selected for the sweetness of glucose, and especially fructose, it can be shown that invert sugar is sweeter, not as sweet, or that it has about the same sweetness as sucrose. Cameron (1947) states that for concentrations below 10% inversion slightly lessens the sweetness of sucrose, while for concentrations above 10% it slightly increases sweetness. Here again the authors wish to emphasize that two entirely different sugar solutions are being discussed.

Factors Affecting Relative Sweetness of Sugars.—*Temperature.*—In discussing the effect of temperature on the sweetness of sugars, a careful distinction must be made between *relative* and *absolute* sweetness. At different temperatures, with sucrose taken as unity times 100 *at each temperature*, the relative sweetness of glucose, galactose, and mannose decreases somewhat with decreasing temperature, but the relative sweetness of fructose decreases dramatically. These data (Tsuzuki and Yamazaki 1953) are shown in Fig. 6.4. The decrease in fructose sweetness parallels the decrease in the concentration of the β-D-pyranose tautomer with thermal mutarotation (Shallenberger 1973).

With increasing temperature, the *absolute* sweetness of glucose and

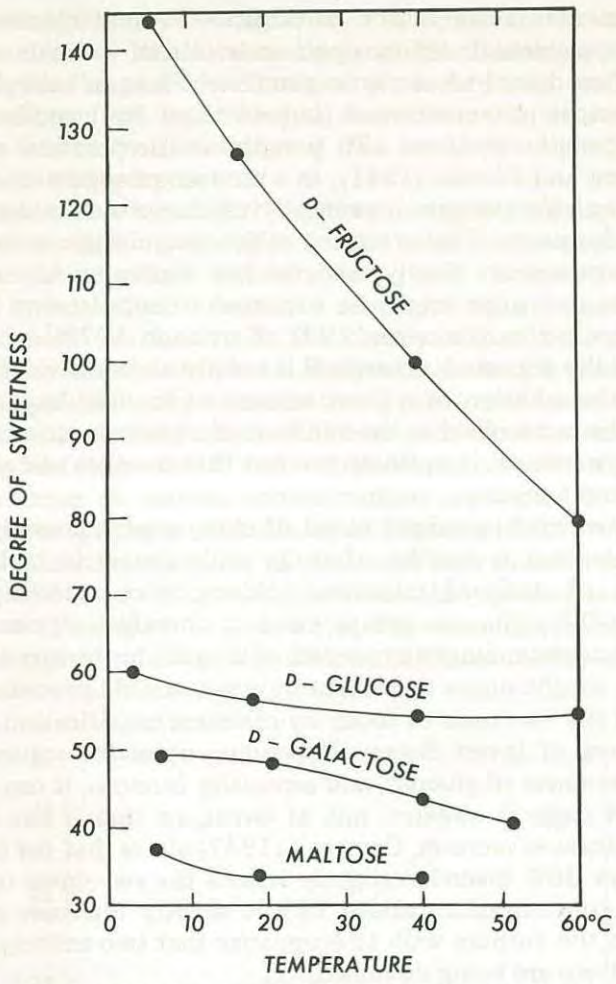


FIG. 6.4. EFFECT OF TEMPERATURE ON THE RELATIVE SWEETNESS OF SUGARS

galactose *increases* (Shallenberger 1963), and this must also necessarily be true for sucrose.

Concentration.—As concentration is increased, the relative sweetness of glucose increases until, as shown in Fig. 6.3, glucose and sucrose are equally sweet at a concentration of about 40%. An uncertainty exists with respect to concentration effect on the relative sweetness of fructose. While Dahlberg and Penzek (1941) and others reported a slight increase in the relative sweetness of fructose with increasing concentration, Tsuzuki and Yamazaki (1953) demonstrated a rather pronounced decrease over the range 3 to 20%.

Viscosity.—The sweetness of sucrose is more difficult to detect in viscous solutions, such as are encountered in foods. Stone and Oliver (1966) found that the thickening agents cornstarch, gum tragacanth, carboxymethylcellulose, methylcellulose and pectin were effective in this respect, but the panel considered the more viscous solutions to be "sweeter." Moskowitz (1970B) found that the relation between viscosity (V) and taste intensity (T) is a power function with a negative slope,

$$T = kV^{-n}$$

where n varies between 0.05 and 0.20.

Technology of Sugars in Foods

Crystallinity.—One major property of sugars in food technology is their crystallinity, which may be either utilized for technological effect or deliberately avoided. In the confectionery industry, for example, production of hard-boiled sweets is accomplished by boiling together sucrose, glucose syrup, acid, color, flavor and water, so that on cooling the mass sets to a "glass" (supercooled liquid). It is essential that this glass should not crystallize or "grain," as it is termed in the food industry, for if it does, the sweet will crumble and disintegrate. The glucose syrup in these formulations functions by inhibiting crystallization of the sugar mass.

Sweetened condensed milk is prepared by evaporating a mixture of fresh milk and added sucrose. In this process the lactose (milk sugar) crystallizes out as the dihydrate; the particle size and distribution can be controlled by a seeding and rapid crystallization technique which ensures that the crystals are small, of a uniform size, and present at the desired concentration. If too little lactose hydrate crystallizes out, the product will be too slimy in texture. On the other hand, if too much is present it will be gritty and resemble sand.

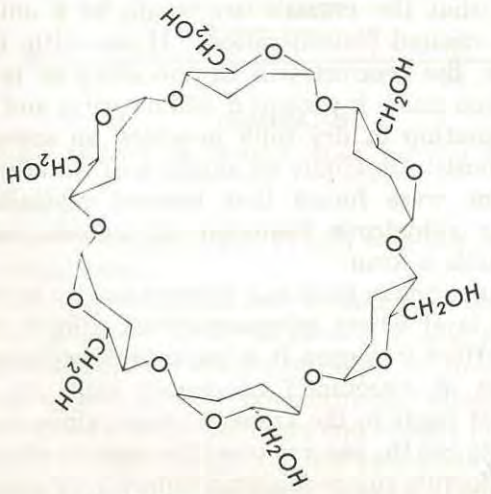
In the preparation of dry milk powders, an acceptable product that would reconstitute rapidly on addition of water was not possible until conditions were found that favored crystallization of the rapidly soluble anhydrous β-anomer of lactose, rather than the relatively insoluble α form.

Sugar also functions in food as a preservative by increasing osmotic pressure to a level where microorganisms cannot reproduce. In assessing this effect in sugars it is important, especially in complex foodstuffs such as sweetened condensed milk, to determine the concentration of sugar in the aqueous phase, since only by lowering the water activity can the sugar prevent the growth of microorganisms.

In jam manufacture sugars function not only by increasing osmotic pressure to a level (around 70% w/v soluble solids) where preserva-

tion against microorganisms is ensured, but also by causing a water-binding or dehydration action, so that the pectin molecules can coalesce and induce a gelling process. In sugar-acid-pectin gels the ratio of these three components is critical to ensure formation of a gel of the proper texture. Imbalance of the ratio will cause a rubbery gel to form; or the liquid may separate, causing "weeping" (syneresis) on storage. While the total sugar concentration must be above a certain level to ensure preservative action, there is a maximum desirable concentration above which sugar is liable to crystallize out on storage. Furthermore, during the boiling process some of the sucrose is hydrolyzed by the action of the citric acid to an equimolar mixture of glucose and fructose (invert sugar). If the inversion of sucrose is insufficient, sucrose may crystallize on storage. If it is too extensive, glucose will crystallize on storage. Manufacturers aim at a final invert sugar concentration of between 20 and 40% for optimum storage properties, but if glucose syrup is present in the formulation the range is probably a good deal wider.

Humectancy.—Yet another technological property of sugar molecules is their water-binding capacity or humectancy. In the case of low molecular weight carbohydrates this may take the form of rapid water absorption from the atmosphere, resulting in sticky, hygroscopic effects. For this reason high D.E. (i.e., low molecular weight) glucose syrups cannot be spray-dried and, if produced in powder form by vacuum evaporation, must be stored in sealed containers. High molecular weight carbohydrates, such as edible gums, dextrins



α-DEXTRIN

and low-D.E. glucose syrups, absorb water without hygroscopic effects, and thus are technologically useful in preventing water migration into or out of packaged foods and assisting the free-flowing properties of food or drink powders. The spiral structure of dextrin molecules also gives rise to an additional advantage, namely the occlusion of small flavor molecules within the spiral helix. This is said to be especially advantageous in the use of Schardinger dextrins (cyclodextrins), where the molecule is able to trap flavor molecules within itself, thus preventing loss of valuable volatiles during food processing.

Analysis of Sugars in Foods

It is convenient for scientists dealing with sugars within the sphere of food technology to divide them broadly into "reducing sugars" and "non-reducing" sugars on the basis of their reaction with Fehling's solution (alkaline cupric tartrate). The former sugars, which possess a free anomeric center, reduce the cupric ion to a red cuprous oxide precipitate. The latter type, of which the common example is sucrose itself, are without action on Fehling's solution. Obviously, after hydrolysis (inversion), sucrose is converted into an equimolar mixture of glucose and fructose that will reduce Fehling's solution; hence determination of the reducing power before and after inversion gives a measure of the sucrose by difference, due allowance being made for the water molecules added during hydrolysis. If the only sugars present are sucrose and invert sugar, the amount of invert sugar is simultaneously determined, but if glucose syrup is also present, the calculation becomes much more complex and the entire analysis may require a chromatographic step.

Polarimetry is also a widely used method of analysis for sugars, and the same principle as outlined in the preceding paragraph can be used to determine sucrose by measuring polarization before and after inversion. Sucrose is extremely labile to acids because of the fructofuranose moiety in its molecule, so that conditions of hydrolysis may be chosen that are mild enough to ensure that sucrose alone in a mixture of any common food oligosaccharides is hydrolyzed.

For routine work in the food industry sugar content may be determined rapidly by refractometry or hydrometry. These methods are highly convenient for quality control, but accuracy is much less than with the analytical techniques, and of course only total soluble carbohydrate can be estimated in this way. For an analytical measure of total carbohydrate many of the modified aldehyde procedures may be employed, the simplest and best being the phenolsulfuric acid technique. In this an aqueous solution of the carbohydrate contain-

ing 10 to 100 μg of carbohydrate is mixed with 5% aqueous phenol, and 5 ml of concentrated sulfuric acid is run in from a fast-delivery pipette; the heat produced assists in the formation of an orange color whose absorbance at λ_{max} at about 490 nm is proportionate to the total carbohydrate concentration. In this technique all polysaccharides are hydrolyzed and effectively react in the same way as monosaccharides, so that a calibration curve for the latter suffices to standardize the method. The cheapness and availability of these reagents and the stability of the resulting colored complex, which may be left standing for at least 1 hr in the laboratory, provide an ideal control method for analysis of carbohydrates in food (Barnett and Tawab 1957; Birch 1963).

For accurate analysis of reducing and non-reducing sugars in foods several points must be considered. First, a variety of oxidation-reduction procedures are available which utilize the fact that sugars with a free anomeric center have an intrinsic reducing ability; however, this is a very common property, which is also true of a number of other food substances, such as the amino acids and ascorbic acid. These impurities must be removed by some clarification procedure. Fortunately, sugars are also intrinsically neutral, and potentially interfering compounds having an acid or base function can be removed by an appropriate ion-exchange procedure (Williams *et al.* 1949).

The extraction of sugars from foods prior to clarification can present a problem, but the use of 80% ethanol generally removes sugars from most foods and effects, in part, an initial clarification, since polysaccharides and proteins are not usually soluble in 80% ethanol. The alcohol must be removed from the extracts, however, since it interferes not only with ion-exchange clarification, but also with the alkaline degradation reaction necessary with the use of Fehling's reagents.

For mixtures of reducing sugars, it is found that their reducing power toward Fehling's solution may not be the same. Glucose and fructose give the same result, but the reducing power of galactose is about 10% lower.

Combinations of volumetric, colorimetric, polarimetric and rapid physical techniques may be used for routine control purposes in the food industry, where standard specifications for a particular product are a major consideration. For a more thorough fundamental understanding of mixtures of carbohydrates, such as are obtained by hydrolysis of complex polysaccharides, chromatography in all its aspects is a common tool. Both paper and thin-layer chromatography are widely used, the former possibly having advantages in terms of

resolution and quantitative convenience and the latter in terms of speed of operation and qualitative convenience.

Routine paper chromatographic procedures for glucose, fructose and sucrose in foods (Shallenberger and Moores 1957) and also for the galactosylsucrose series of oligosaccharides (Shallenberger and Moyer 1961) have been used to great advantage in determining individual sugars in foods for a number of years. Gas-liquid chromatography (g.l.c.) of sugars has developed during the last 15 years, and is obviously to be a routine tool of the future, with a variety of specialized uses (Birch 1973). Although the sugars themselves are not volatile, they may be converted to volatile derivatives, the most popular of which appear to be the trimethylsilyl ethers (TMS ethers). Homologous series of oligosaccharides such as are obtained in glucose syrup mixtures can be separated by g.l.c. as their TMS derivatives with retention times between 10 and 140 minutes. With modern columns and modern techniques, derivatives with molecular weights up to 3000 are said to be separated in this way (Beadle 1969).

For fundamental studies of carbohydrate structures nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry and infrared or U.V. absorption may be used alone or in combination with chromatographic separation, especially g.l.c. procedures. Such sophisticated instruments are now entering the research laboratories of the food industry but are not of much use on the factory floor except in the case of simple types of spectrophotometer.

MEDICINE

Absorption of Carbohydrate and the Role of Blood Sugar in Health and Disease

As mentioned in Chapter 5, diabetics suffer from the combined effects of rapid absorption of carbohydrate and rapid uncontrolled rise in blood glucose level. Both blood and urine of diabetics may be examined chromatographically for the presence of different types of sugar, as is also done in the diagnosis of other inherited metabolic disorders of carbohydrate metabolism, such as galactosemia. In the latter disease patients lack the enzyme glucose 1-phosphate galactose 1-phosphate uridyl transferase, so that galactose is unable to be metabolized through normal channels (e.g. transformation to glycogen in the liver); hence galactose 1-phosphate builds up to intolerable levels. Unless galactosemia is detected early in infancy by utilizing chromatographic procedures or specific galactose oxidase enzymes for identification of galactose in blood and urine, the disease may be

fatal. Treatment consists of removing galactose and lactose from the diet. If not fatal, the disease leads to cataract and mental retardation.

Other diseases in which simple enzyme systems may be absent from the liver are the glycogen storage diseases. These may be characterized by massive deposits of glycogen in the liver. There are at least 4 different types of the disease; like galactosemia, it is very rare, probably one birth in 30,000 to 50,000 being an estimate of its occurrence.

In the diseases of malabsorption certain types of dietary carbohydrate are unavailable due to the absence of a particular digestive enzyme (e.g., disaccharidases) or pathological condition of the brush border region of the small intestine (Chapter 5). In these it is frequently necessary to confirm diagnosis by using either xylose or 3-*O*-methyl-D-glucose as markers of the absorptive process. The rare food sugar, xylose, is only partly metabolized because at least 40% of it is excreted unchanged in the urine; hence the rate at which it is excreted after ingestion is an indication of the efficiency of the absorptive process. Most recent radioautographic work (Stirling *et al.* 1972) utilizing galactose-^{14}C and galactose-^{3}H has clearly demonstrated that in cases of glucose-galactose malabsorption the inborn error of transport is a persistent reduction in the number of functioning sugar carriers at the microvilla membrane.

Although rare food sugars are either readily excreted unchanged or are otherwise metabolized to glucose in the liver, a number of these substances appear to be normal constituents of mammalian body fluids, though at a very low concentration. Fasting levels of sucrose, lactose and raffinose in human serum are probably about one-thousandth of the blood glucose concentration; in urine, on the other hand, lactose concentration is one-hundreth of the serum glucose level (Nakamura and Tamura 1972; Toseland 1968). After simultaneous administration of lactose, maltose and sucrose the urine level of lactose reaches 10% of the fasting blood glucose concentration (i.e., about 5 mg lactose per 100 ml urine). Other food sugars that are normal constituents of human urine are raffinose, sucrose, maltose, fucose, arabinose, galactose, xylose, mannoheptulose and ribose. Stachyose should probably be added to the list. Like raffinose, the human does not seem to possess the enzymes to hydrolyze stachyose, and if it is hydrolyzed, it is another source of dietary galactose. In the urine, those sugars listed may be present at considerably higher concentrations than the urinary glucose level.

In certain disease states when the feeding of sugars that are metabolizable to glucose is contraindicated (e.g., in certain complications of diabetes) physicians have tried the experiment of infusing

the rare food sugar, mannose, and the sugar alcohol, xylitol. Both substances resulted in considerable physiological disorder including deeply orange-colored urine, feelings of nausea, stupor and coma (Birch 1971A, B; Wood and Cahill 1963; Thomas *et al.* 1970). On the other hand the $\alpha, 1 \rightarrow 6$ glucosidically linked polymer dextran has been used as a plasma extender; it would appear that maltodextrins may have similar value in this respect, although a limited existence for these may be expected because of the presence of a circulating amylase.

Clinically Designed Carbohydrate Foods

Whenever inborn deficiencies of metabolism result in the inability of patients to cope metabolically with a specific sugar or mixtures of sugars there appears to be a case for the design of special dietary products (Birch 1971A, B; Veeger 1972). Carbohydrate modification is probably one of the most important aspects of clinically designed foods in general, since either over- or under-consumption of calories is a widespread problem. Under-consumption of carbohydrate normally occurs only in famine, but the deficiency has been detected during athletic performance when all energy reserves are depleted and the condition of ketosis results. Over-consumption of carbohydrate leads to the more common manifestations of obesity, diabetes, and related nutritional disorders.

Among those foods clinically designed for use in cases of carbohydrate intolerance, low-lactose preparations are most common; manufacturers in the U.K. and U.S. are supplying these to physicians for lactose-intolerant patients and galactosemics. The lactose in these preparations may be replaced with sucrose, maltose, glucose syrup, maltodextrins or dextrose. Rarer types are the low-fructose foods for use by fructose intolerants and conversely the fructose-formula type used by glucose-galactose malabsorbers. Humanized baby foods contain a higher proportion of lactose, which may be the only carbohydrate added. In renal failure physicians have the unique problem of feeding the entire daily calorific requirement to the patient without added protein and without causing feelings of nausea in the patient, as would result from excessive consumption of sweet syrups. This is accomplished by the use of low-D.E. glucose syrups sweetened with saccharin.

Sugars and Heart Disease

Yudkin (1971) believes that sucrose intake in populations can be correlated with the incidence of heart disease. However, the experimental substantiation of this belief must rest largely on nutritional tests on animals, which vary in their significance to man, not least because the metabolic responses of carnivores, herbivores and omni-

vores differ. Much of the early experimental work with animals was on the cholesterol level in the blood, which is more likely to be higher in those who are prone to coronary heart disease than in those who are not. More recently it has been suggested that there is a closer relationship to the triglyceride level (Yudkin 1971), but age and sex differences in mammals may cause considerable differences in experimental results (Birch *et al.* 1973) when serum and tissue lipid responses are examined in relation to dietary carbohydrate intake. Although most of the experimental work with rats has indicated that sucrose causes higher serum cholesterol and triglyceride levels than starch when either substance is fed as the sole dietary carbohydrate, these results have largely been obtained on male rats, which are selected for study because they are steadier than females in their metabolic response. Whereas female rats respond with generally increased tissue lipid levels as the molecular weight of the dietary carbohydrate increases (Birch and Etheridge 1973), no such results are obtained with male rats. In humans and primates (MacDonald 1966), although sucrose causes an increase in the fasting triglyceride level of males, the opposite effect seems to occur in females; a natural estrogen appears to provide the protective effect. These results also depend on the concentration and type of dietary fat, and the rise of cholesterol level in the serum after ingestion of particular types of dietary carbohydrate depends, too, on the concentration of cholesterol in the diet.

Elevation of serum lipid levels could contribute to atherosclerosis by aiding the formation of the atheromatous deposit on the interior of the arterial wall. However, there is now reason to believe that there are more relevant changes in the blood of coronary-prone individuals than the lipid changes described above. In particular, attention has been drawn to the blood-clotting mechanism and serum insulin level. About one-third of a group of 13 men given a diet rich in sucrose showed both an increase in serum insulin level and blood platelet adhesiveness (Szanto and Yudkin 1969), the latter phenomenon possibly initiating the clotting process.

Clear structural differences in the ability of different sugars to cause heart disease are difficult to achieve unequivocally, because of the number of dietary controls and subjective restrictions required. While there is a public demand for sweetness, it seems premature to suggest the substitution of artificial sweeteners for dietary sucrose. Indeed, this could lead to an even greater nutritional hazard.

Dietary Fiber

Dietary "crude fiber" is defined chemically as the organic residue remaining after the food material has been extracted under standard

conditions with petroleum ether, boiling dilute sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether. It consists largely of cellulose together with a little lignin, but also contains some pentosans (hemicelluloses) and mucilages (a wide variety of types of polysaccharide including galactomannans, glucomannans, neutral and acidic polysaccharides). Its composition varies according to its source and even the chemical form and degree of crystallinity of the cellulose vary in the same way. Hence assignment of nutritional properties to particular molecular entities in this class of substance is still not possible.

Nutritionists have traditionally regarded fiber as the roughage of the diet, stimulating peristaltic movement of the intestine, but in recent years a new hypothesis has emerged. By assisting the passage of food masses along the alimentary canal, fiber decreases intestinal transit time. Thus the average intestinal residence time for Westerners may be between 60 and 80 hr, whereas that of many African communities who live on a less-refined (higher-fiber) diet is around 48 hr. Long intestinal residence times result in hard, dry stools that require considerable intestinal strain and pressure for passage (Painter and Burkitt 1971), so that diverticulitis of the colon has a relatively high incidence in Western Communities, whereas in African communities it is practically unknown. The same basic explanation is said to be applicable to the incidence of appendicitis, hemorrhoids, varicose veins, and many other disorders.

The contention (Trowell 1972) that dietary fiber can lower the serum cholesterol level in man is of especial interest. Whole cereal grains contain 2 gm of fiber/100 gm; leguminous seeds are richer, containing 3 to 5 gm/100 gm. In a recent experiment in India male volunteers ate 247 gm of Bengal gram (chick peas) and thereby received 16 gm of fiber a day, while also consuming considerable butter fat (156 gm/day). They showed a marked reduction in serum cholesterol level, from 206 to 160 mg, but it was necessary to consume the diet for 20 weeks to achieve the maximum hypocholesteremic effect. In African communities in which 6 to 12 gm of fiber per day per individual are ingested, there is very little coronary disease. The fiber theory has been criticized (Dodds *et al.* 1972) but largely on the grounds of faulty epidemiological evidence in comparing modern incidence with that in the nineteenth century.

Therapeutic Uses of Sugars

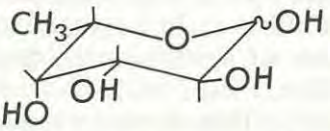
Probably the historic belief that sugars are among the most metabolically inert components of the diet is founded on the high concentrations that are normally required to produce an observable nutritional effect. For example, in the glucose tolerance test they

are fed at around 1 gm/kg of body weight, while in dietary studies the level of administration usually lies between 60 and 90% of the dietary intake.

Therapeutic uses of the sugars are scant and the few reported examples are not always sugars themselves, but their derivatives. The best-known example is vitamin C, which is an unsaturated lactone derivative of the aldonic acid obtained from L-gulose. Although well-known for its alleged anti-common cold properties (Pauling 1972), it has also been implicated as an anti-carcinogen. The excessive use of this vitamin, however, could conceivably result in a rise of serum oxalate level (Sinclair 1972).

Considerable protection against dental caries has been claimed for the use of the calcium sucrose phosphates (Anon. 1967) which, when incorporated into normal confectionery at a level of about 2 to 3% of the total sugars, have reduced the incidence of caries in Australian school children. The mechanism of action of these agents is still not proved, but may involve a metabolic block of the mouth micro-organisms, which utilize the dextran-sucrase enzymic system for the production of dental plaque. Plaque contains a 1-6-glucosidically linked polymer that coats the surface of the teeth and appears to be associated with the carious lesions themselves.

In cancer research some of the fluorinated carbohydrates may offer therapeutic possibilities as antimetabolites, while natural pentoses (hemicelluloses) can successfully regress experimental tumors. The deoxy sugar, L-fucose, is able to suppress the growth of a transplanted mammary tumor in rats (Seltzer *et al.* 1969).



The pentose, ribose, has been used to induce hypoglycemia (Ginsburg *et al.* 1970) and in this it differs from other pentoses (xylose and arabinose), which have no effect on the blood glucose level. The reasons underlying this basic difference have still not been explored, but the massive dose of ribose required for such an effect (1 gm/kg) seems to induce the usual diarrhea and borborygmii of the rare food sugars along with the hypoglycemic response.

The disaccharide lactulose (4-*O*-β-D-galactopyranosyl fructose) has been used to induce an increase in the fecal population of *Lactobacillus bifidus* and to treat constipation, especially in the elderly. This unabsorbed sugar seems to act as an extremely mild purgative and because of its stimulation of the colonic lactobacilli it reduces those organisms that produce ammonia. It is therefore of value in

treating the condition known as chronic hepatoportal encephalopathy, in which nitrogenous substances formed in the colon bypass the liver and affect the brain.

BIBLIOGRAPHY

ANON. 1967. Research: Food additive to reduce dental caries. Australian Sugar J. *59*, 98-101.

BARNETT, A. J. G., and TAWAB, G. A. 1957. A rapid method for the determination of lactose in milk and cheese. J. Sci. Food Agr. *8*, 437.

BEADLE, J. B. 1969. Gas chromatographic determination of starch hydrolyzate saccharide distribution through maltoheptaose. J. Agr. Food Chem. *17*, 904-906.

BIRCH, G. G. 1963. Spectrophotometric determination of glucose content in glucose derivatives. Proc. Assoc. Clin. Biochem. *2*, 201-202.

BIRCH, G. G. 1963. The characterization of acid-hydrolyzed corn syurps; a theoretical appraisal. J. Food Technol. *3*, 159-163.

BIRCH, G. G. 1971A. Metabolic effects of rare food sugars. Lancet *7739* (2), 1419-1420.

BIRCH, G. G. 1971B. Carbohydrate disorders: Causes, effects and the case for dietary foods. Food World *6*, 9.

BIRCH, G. G. 1972. Lactose: One of Nature's paradoxes. J. Milk Food Technol. *35*, 32-34.

BIRCH, G. G. 1973. Gas-liquid chromatography of food carbohydrates with special reference to trimethylsilyl derivatives. J. Food Technol. *8*, 229-246.

BIRCH, G. G., COWELL, N. D., and YOUNG, R. H. 1972. Structural basis of and interaction between sweetness and bitterness in sugars. J. Sci. Food Agr. *23*, 1207-1212.

BIRCH, G. G., and ETHERIDGE, I. J. 1973. Chemical and physiological properties of glucose syrup components. Die Staerke. *25*, 235-238.

BIRCH, G. G., ETHERIDGE, I. J., and GREEN, L. F. 1973. Short term effects of feeding glucose syrup fractions and dextrose in rats. Brit. J. Nutr. *29*, 87-93.

BROOK, M. 1970. Sugar substitutes and their significance for dental health. Dental Health. *9*, 46-52.

CAMERON, A. T. 1947. The taste sense and the relative sweetness of sugars and other substances. Sci. Report *9*. Sugar Research Foundation, New York.

DAHLBERG, A. C., and PENZEK, E. S. 1941. The relative sweetness of sugars as affected by concentration. N.Y. State Agr. Exp. Sta. Bull. *258*.

DODDS, C., FISCHER, N., GREENWOOD, C. T., and HUTCHINSON, J. B. 1972. Effects of dietary fiber. Brit. Medical J. *3*, 472-473.

GREEN, L. F. 1971. The balance of natural and synthetic sweeteners in food. *In* Sweetness and Sweeteners, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.

HERRMANN, J. 1973. Evaluation and analysis of sensory qualities of food and calculation of their changes. VI. Calculation of the relative sweetness of sugar mixtures. Nahrung *17*, 11-22.

HODGE, J. E., RENDLEMAN, J. A., and NELSON, E. C. 1972. Useful properties of maltose. Cereal Sci. Today *17*, 180-188.

MACDONALD, I. 1966. Influence of fructose and glucose on serum lipid levels in men and pre- and postmenopausal women. Am. J. Clin. Nutr. *18*, 369-373.

MOSKOWITZ, H. R. 1970A. Ratio scales of sugar sweetness. Perception and Psychophys. *7*, 315-320.

MOSKOWITZ, H. R. 1970B. Taste intensity as a function of stimulus concentration and solvent viscosity. J. Text. Studies *1*, 502-510.

NAKAMURA, H., and TAMURA, Z. 1972. Gas chromatographic analysis of

mono- and disaccharides in human blood and urine after oral administration of disaccharides. Clin. Chim. Acta *39*, 367-381.
PAINTER, N. S., and BURKITT, D. P. 1971. Diverticular disease of the colon: a deficiency disease of Western civilization. Brit. Medical J. *5759 2*, 450-454.
PAINTER, T. J. 1963. Random depolymerizations in which the lower oligosaccharides are protected from further scission. J. Chem. Soc. 779-783.
PAULING, L. 1972. Vitamin C and the Common Cold. W. H. Freeman and Co., San Francisco.
SHALLENBERGER, R. S. 1963. Hydrogen bonding and the varying sweetness of the sugars. J. Food Sci. *28*, 584-589.
SHALLENBERGER, R. S. 1973. Sugar structure and taste. *In* Carbohydrates in Solution, R. F. Gould (Editor). Advances in Chemistry Series *117*, 256-263.
SHALLENBERGER, R. S., and ACREE, T. E. 1971. Chemical structure of compounds and their sweet and bitter taste. *In* Handbook of Sensory Physiology, IV. Chemical Senses, 2. Taste, L. M. Beidler (Editor). Springer Verlag, Berlin.
SHALLENBERGER, R. S., and MOORES, R. G. 1957. Quantitative determination of reducing sugars and sucrose separated by paper chromatography. Anal. Chem. *29*, 27-29.
SHALLENBERGER, R. S., and MOYER, J. C. 1961. Relation between changes in glucose, fructose, galactose, sucrose and stachyose and the formation of starch in peas. Agr. Food Chem. *9*, 137-140.
SELTZER, M. H. *et al.* 1969. The effect of L-fucose on rat mammary tumor growth. Growth, *33*, 353-359.
SINCLAIR, H. M. 1972. The rationale of health foods. *In* Health and Food, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.
STIRLING, C. E., SCHNEIDER, A. J., WONG, M. D., and KINTER, W. B. 1972. Quantitative radiography of sugar transport in intestinal biopsies from normal humans and a patient with glucose-galactose malabsorption. J. Clin. Invest. *51*, 438-451.
STONE, H., and OLIVER, S. 1966. Effect of viscosity on the detection of relative sweetness intensity in sucrose solutions. J. Food Sci. *31*, 129-131.
STONE, H., and OLIVER, S. M. 1969. Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. J. Food Sci. *34*, 215-222.
SZANTO, S., and YUDKIN, J. 1969. The effect of dietary sucrose on blood lipids, serum, insulin, platelet adhesiveness and body weight in human volunteers. Postgrad. Med. J. *45*, 602-607.
THOMAS, D. W., EDWARDS, J. B., and EDWARDS, R. G. 1970. Examination of xylitol. New Engl. J. Med. *283*, 437.
TOSELAND, P. A. 1968. Specific determination of plasma and urinary lactose. J. Clin. Pathol. *21*, 112-113.
TROWELL, H. 1972. Fiber: a natural hypocholesteremic agent. Am. J. Clin. Nutr. *25*, 464-465.
TSUZUKI, Y., and YAMAZAKI, J. 1953. On the sweetness of fructose and some other sugars, especially its variation with temperature. Biochem. Z. *323*, 525-531.
WILLIAMS, K. T., POTTER, E. F., BEVENUE, A., and SCURZI, W. R. 1949. Study of clarification methods in the determination of sugars in white potatoes. J. Assoc. Official Agr. Chem. 698-705.
WOOD, F. C., and CAHILL, G. F. 1963. Mannose utilization in man. J. Clin. Invest. *42*, 1300-1312.
VEEGER, W. C., 1972. Clinically designed foods. *In* Health and Food. G. G. Birch *et al.* (Editors). Applied Science Publishers, London.
YAMAGUCHI, S., YOSHIKAWA, T., IKEDA, S., and NINOMIYA, T. 1970. Studies on the taste of some sweet substances. I. Measurement of relative sweetness. Agr. Biol. Chem. (Tokyo) *34*, 181-186.
YUDKIN, J. 1971. Sucrose in the aetiology of coronary thrombosis and other diseases. *In* Sugar, J. Yudkin *et al.* (Editors). Butterworths, London.

Nonenzymic Browning Reactions

Many foods are subject to the so-called "nonenzymic" browning reactions, either during processing or upon storage of the finished product. While it is not necessarily true in all cases, sugars are usually the principal nonenzymic browning reactants. The reactions are accompanied by flavor development, texture alteration, and the initiation of yellow, red, brown, and black coloration. Food nutritive value is invariably altered.

Many foods have characteristic color and flavor generated by purposely induced browning reactions. These are relatively high-temperature reactions employed, for example, in the generation of caramel from sugars, roasting, deep-fat frying, and baking operations. Other foods, processed by dehydration or concentration, are subject to browning reactions wherein color and flavor develop; this is undesirable, since the usual color and flavor standard of identity for the product is altered. Such reactions may occur during storage of the food product at relatively low temperatures. The "stackburn" found in warehoused, but undercooled canned food products is also due to these reactions (Shallenberger and Pederson 1961).

The nonenzymic browning reactions are a diverse group of dehydration, fragmentation and polymerization reactions whose chemistry and kinetics have never been fully described, not even for a single case. When the reactions occur in the absence of nitrogenous compounds, they are described as *caramelization* reactions. When they occur in the presence of nitrogenous compounds, especially primary and secondary amines, they are called *carbonyl-amine* or *Maillard* reactions (Hodge 1953).

In the initial stages the major chemical events that occur either during caramelization or carbonyl-amine browning have been established. The later stages of the reactions are ill-defined. The sequence of chemical events that predominate depends on the environment; the temperature, pH, reactant concentration, nature of the reactants, and time have marked effects.

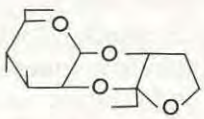
CARAMELIZATION REACTIONS

Under anhydrous conditions upon the application of heat, or at high concentration in dilute acid solutions, the initial stages of the caramelization reaction are characterized by the formation of

anhydro sugars. Glucose has been reported to yield glucosan (1,2-anhydro-α-D-glucose) and levoglucosan (1,6-anhydro-β-D-glucose). The two anhydro glucose compounds are readily distinguished by their specific rotation. Values reported are +69° and -67°, respectively. The former compound is fermented by yeast and the latter is not. The dimerization of these compounds probably accounts for the various reversion disaccharides, such as gentiobiose and sophorose, found when glucose is melted.

Fructose initially yields levulosan (2,3-anhydro-β-D-fructofuranose) with a specific rotation of +18.6° (Pictet and Reilly 1921). Dimerization leads to the entire series of di-D-fructose anhydrides mentioned in Chapter 4.

When sucrose is heated at about 200° simultaneous "hydrolysis" and dehydration occur, apparently followed by rapid dimerization of the products, so that a series of compounds characterized by isosacchrosan (corresponding to sucrose minus one molecule of water) is formed. Isosacchrosan was shown by Pictet and Stricker (1924) to be 1,3′; 2,2′-dianhydro-α-D-glucopyranosyl-β-D-fructofuranose:



ISOSACCHROSAN

Isosacchrosan has no sweetness, but does have a mildly bitter taste.

In dilute solutions of reducing sugars, the initial stages of the caramelization reactions parallel the events described for the enolization, isomerization, dehydration and fragmentation reactions (Chapter 4). Subsequently, polymerization reactions occur, which lead to the formation of pigments similar to those formed in caramelization reactions at either higher temperatures or in more concentrated solution.

The classic caramelization reaction is the phenomenon exhibited by sucrose when subjected to heat, although commercial caramels are produced from glucose syrups (Linner 1973). When sucrose is melted at 160° and kept at that temperature, glucose plus fructose anhydride (levulosan, $C_6H_{10}O_6$) is generated.

At 200°, Shumaker and Buchanan (1932) observed 3 distinct endothermic events during sucrose caramelization. After the sucrose had melted, foaming began and then stopped after 35 min. The weight loss was 4.5%, which corresponds to the loss of one molecule

of water per molecule of sucrose and is thus analogous to the formation of anhydrides such as isosacchrosan.

A second stage of foaming soon began which lasted for 55 min. The weight loss from the system was 9%, which agrees well with the average molecular formula for the pigment *caramelan* (Cunningham and Dorée, 1917), or

$$2C_{12}H_{22}O_{11} - 4H_2O = C_{24}H_{36}O_{18}$$

which requires a weight loss of 10.5%.

Isolated caramelan, which is soluble in water and ethanol, melts at 138° (Miroshnikova *et al.* 1970) and has a bitter taste.

A third stage of foaming which began after about 55 min was characterized by *caramelen* formation. The average molecular weight of the pigment corresponds to the loss of 8 molecules of water from 3 molecules of sucrose

$$3C_{12}H_{22}O_{11} - 8H_2O = C_{36}H_{50}O_{25}$$

and a weight loss of 14%. Caramelen is soluble only in water and has a melting point of 154°.

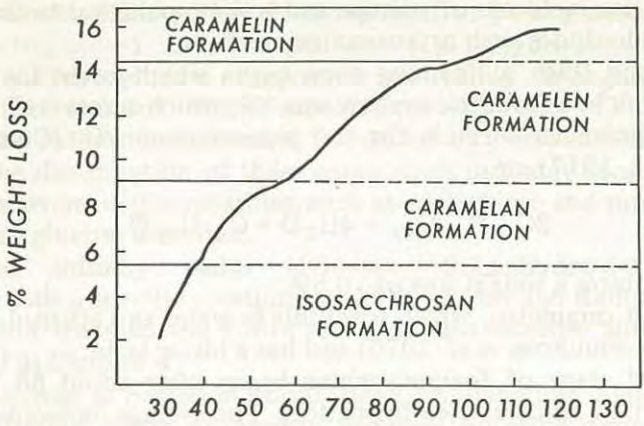
Continued heating leads to the formation of *humin*, a dark infusible mass of high molecular weight. Janacek (1939) and others place the average molecular formula at about $C_{125}H_{188}O_{80}$. A diagrammatic depiction of caramelization events, taken from Shumaker and Buchanan (1932) is shown in Fig. 7.1. *Caramelin* is the technological term for humin. Clearly, sugar caramels vary in properties and constitution depending upon the time and temperature of heating; and while the caramelization events presented in Fig. 7.1 are characteristic of sucrose caramelization, they are by no means the only phenomena to occur.

Varying amounts of the so-called "reversion" sugars can be found in the preparations, and sugar fragmentation and dehydration products account for the characteristic caramel flavor. Among these compounds are diacetyl, acetic acid, and formic acid. Only two degradation products, acetylformoin (4-hydroxy-2,3,5-hexanetrione) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, are reported to possess typical caramel flavor (Jurch and Tatum 1970). Hodge (1967) proposed that compounds that have caramel odor have the groupings

$$CH_3-\overline{C}=C(OH)-\overline{C}=O$$

AND

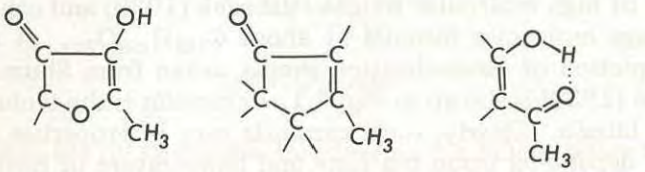
$$CH_3-\overset{O}{\overset{\|}{\overline{C}}}-\overline{C}=\overline{C}(OH)$$



Shumaker and Buchanan (1932)

FIG. 7.1. CARMELIZATION OF SUCROSE

and that the structures may be either *O*-heterocyclic or alicyclic, or may possess an intramolecular hydrogen-bonded chelate ring:



HETEROCYCLIC ALICYCLIC HYDROGEN-BONDED

Some typical flavors of caramelization substances are given in Table 7.1.

The caramel pigments are probably polymers with pyran nuclei (O'Colla *et al.* 1962) with the predominant linkage of the 1,6 type. The pigments are also colloidal and have varying particle size. Sapronov *et al.* (1969) list the average radii of the caramel compounds, corresponding to caramelan, caramelen, and caramelin at 0.46, 0.95, and 4.33 nm, respectively.

As colloidal substances, the caramel pigments have an isoelectric point that varies with the method of preparation. They are positively charged at pH values more alkaline than their isoelectric point, and negatively charged at pH values more acid than their isoelectric point (Greenshields *et al.* 1969).

In addition to hydroxyl groups of varying basicity, the caramel pig-

ments possess carbonyl, carboxyl, and enolic groups (Miroshnikova *et al.* 1970). Judging from the results obtained by Plunguian and Hibbert (1935), phenolic hydroxyl groups are also present, which seems to explain why iron intensifies the caramel color. The ability of iron and phenolic substances to react to form blue to black coloration is well-known in the food industry.

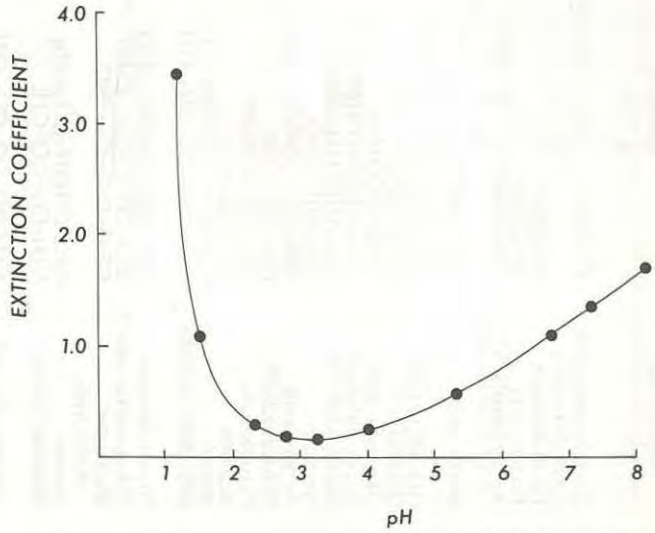
The mechanism of polymerization of the sugar fragmentation and dehydration products seems to be analogous to the aldol condensation reaction. Aldol condensation is typical of simple aldehydes and ketones. Acetaldehyde, upon standing, dimerizes to form aldol, which then loses a molecule of water to form crotonaldehyde:

$$2CH_3CHO \longrightarrow CH_3CH(OH)CH_2CHO \longrightarrow CH_3CH{=}CHCHO$$

ACETALDEHYDE ALDOL CROTONALDEHYDE

Continuation of the reaction yields colored polymers of high molecular weight and increasing proportions of carbon by progressive dehydration.

The caramelization reaction is autocatalytic (Doss and Ghosh 1949) and increasing temperatures not only increase the reaction rate but also alter the qualitative nature of the pigments. The effect of pH is dramatic. The reaction rate at pH 8 is ten times greater than that at pH 5.9 (Ledon and Lananeta 1950). The effect of pH on the



Kröner and Kothe (1939)

FIG. 7.2. EFFECT OF pH ON THE INTENSITY OF COLOR DEVELOPED IN HEATED GLUCOSE SOLUTIONS

TABLE 7.1

FLAVORS OF CARBOHYDRATE CARAMELIZATION AND DEHYDRATION PRODUCTS

Compound	Formula	Color	Odor	Taste
		Acyclics		
Carbon monoxide, dioxide	CO, CO_2	None	None	None
Formaldehyde	$H \cdot CHO$	None	Stinging, suffocating	...
Formic acid	$H \cdot COOH$	None	Painful	Sour
Acetaldehyde	$CH_3 \cdot CHO$	None	Penetrating, suffocating	...
Acetic acid	$CH_3 \cdot COOH$	None	Pungent, painful, vinegar, if dilute	Sour
Glycolaldehyde	$HO \cdot CH_2 \cdot CHO$	None	None	Sweet
Glyoxal	$CHO \cdot CHO$	Yellow	Pungent	Sweet
Glyoxylic acid	$CHO \cdot COOH$	...[1]	Suffocating	Sour
Lactic aldehyde	$CH_3 \cdot CHOH \cdot CHO$	None	None	Bitter
Lactic acid	$CH_3 \cdot CHOH \cdot COOH$	None	None	Sour
Acrolein	$CH_2 : CH \cdot CHO$	None	Acrid, scorched fat	...
Acrylic acid	$CH_2 : CH \cdot COOH$	None	Acrid	Sour
Pyruvaldehyde	$CH_3 \cdot CO \cdot CHO$	Yellow	Pungent, burning	...
Pyruvic acid	$CH_3 \cdot CO \cdot COOH$	Yellow	Acetic	Sour
Acetone	$CH_3 \cdot CO \cdot CH_3$	None	"Characteristic"	Sweet, burning
Acetol	$CH_3 \cdot CO \cdot CH_2 \cdot OH$	None	"Characteristic"	Sweet, burning
Dihydroxyacetone	$HO \cdot CH_2 \cdot CO \cdot CH_2 \cdot OH$	None	"Characteristic," pleasant	Sweet, cooling
Glyceraldehyde	$HO \cdot CH_2 \cdot CHOH \cdot CHO$	None	None	Sweet
Triose-reductone	$HO \cdot CH : C(OH) \cdot CHO$	None	None	Sour
Mesoxalic dialdehyde	$CHO \cdot C(OH)_2 \cdot CHO$	Yellow	Burnt sugar, when heated	...
Acetoin	$CH_3 \cdot CHOH \cdot CO \cdot CH_3$	None	Pleasant[2]	Sweetish[2]
Diacetyl	$CH_3 \cdot CO \cdot CO \cdot CH_3$	Green-yellow	Quinonic, buttery, chlorine-like	Sharp
Hydroxydiacetyl	$CH_3 \cdot CO \cdot CO \cdot CH_2 \cdot OH$	Orange-yellow	Sweetish	...
C-Methyl triose-reductone	$CH_3 \cdot CO \cdot C(OH) : CH \cdot OH$	None	...	...
Levulinic acid	$CH_3 \cdot CO \cdot (CH_2)_2 COOH$	None	None	Sour

Cyclics

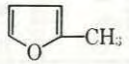
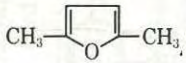
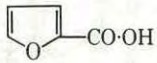
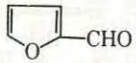
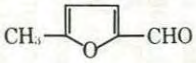
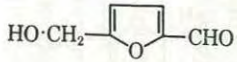
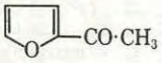
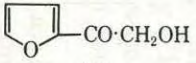
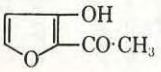
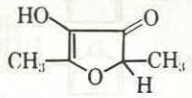
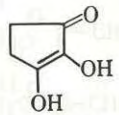
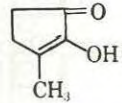
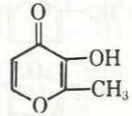
Furan		None	Ethereal	...
2-Methyl furan		None	Ethereal	...
2,5-Dimethyl furan		None	"Characteristic"	...
Furfuryl alcohol		None	"Characteristic"	Bitter
2-Furoic acid		None	Stinging	Sour
2-Furaldehyde		None	"Characteristic"	Sweet
5-Methyl-2-furaldehyde		None	Like 2-furaldehyde	...
5-(hydroxymethyl)-2-furaldehyde		None	None	Bitter, astringent
2-Furyl methyl ketone		None	Pleasant, ketonic	Burning, sweetish
2-Furyl hydroxymethyl ketone		None	None	Burning, sweetish
Isomaltol		None	Burnt, pungent, fruity	Sour, sweet, fruity

TABLE 7.1 (*Continued*)

Compound	Formula	Color	Odor	Taste
4-Hydroxy-2,5-dimethyl-3(2 *H*)-furanone		None	Fragrant, fruity caramel, burnt pineapple	Burning, sweet
Reductic acid		None	None	Sour
1-Methylcyclopentenol(2)-one-(3)		None	Fragrant, burnt, licorice	Sweet
Maltol		None	Fragrant, caramel	Bitter, sweetish

Source: Hodge (1967).

[1] Colorless as the hydrated form.

[2] Traces of diacetyl usually interfere.

stability of glucose, as measured by color development, is shown in Fig. 7.2 (Kröner and Kothe 1939). It is obvious from this figure that caramelization reactions are qualitatively different at different pH values; the results shown are the source of the ubiquitous statement that glucose is intrinsically stable at pH 2.8. This is true, of course, only with respect to the intensity of color developed in glucose solutions.

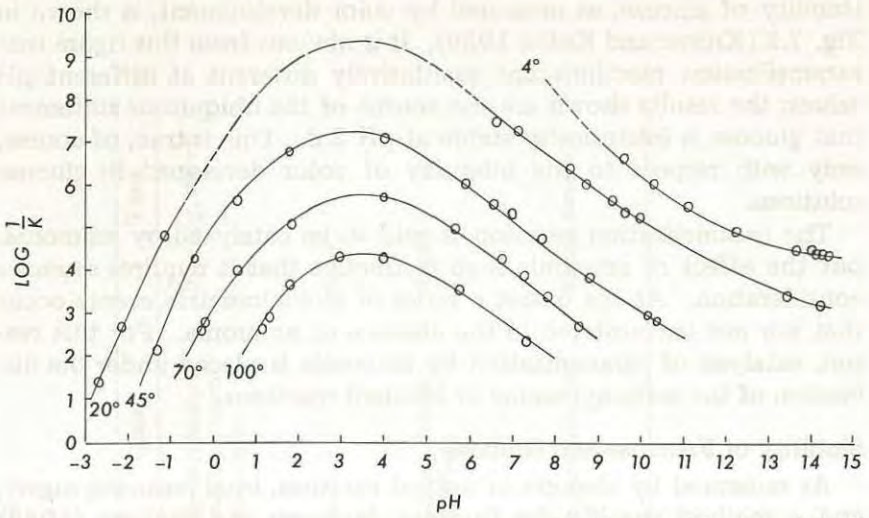
The caramelization reaction is said to be catalyzed by ammonia, but the effect of ammonia is so distinctive that it requires separate consideration. At the outset a series of stoichiometric events occur that are not encountered in the absence of ammonia. For this reason, catalysis of caramelization by ammonia is placed under the discussion of the carbonyl-amine or Maillard reactions.

Stability of Fructose and Glucose

As measured by changes in optical rotation, total reducing sugars, and a method specific for fructose, Mathews and Jackson (1933) found that fructose is most stable at pH 3.3. Their results, emphasizing the qualitative difference in reactions at different pH values showed that in acid solution fructose is dehydrated to form levulosans and that in alkaline solutions the familiar Lobry de Bruyn-van Ekenstein transformation occurs. Since at pH values greater than 3 the transformation is favored, while below this value dehydration is predominant, a pH value of 7 can be considered a highly alkaline condition with respect to the maximum stability of fructose. As judged from the results of Kröner and Kothe (1939), this is also true for glucose.

The stability of fructose is independent of temperature, as shown in Fig. 7.3, where the reciprocal of the polarization velocity constant is plotted against pH. In addition to being independent of temperature, fructose stability at pH 3.3 is also independent of concentration; however, the temperature coefficients for both the acid and alkaline decomposition were found to be uncommonly high. Instead of the usual doubling or trebling of the rate for each 10° increase in temperature, fructose decomposition increased from 5 to 10 times for each 10° rise in temperature.

McDonald (1950), using the polarization technique of Mathews and Jackson (1933), found that glucose has an optimum stability at pH 4 (pH 3 to pH 5), and pointed out that values of k, or polarization velocity constants, are independent of the reaction taking place at different pH values. Glucose stability, as measured by this technique, is also independent of temperature and presumably of concentration.



Mathews and Jackson (1933)

FIG. 7.3. VARIATION IN THE STABILITY OF FRUCTOSE AT VARIOUS TEMPERATURES AND VARIOUS pH VALUES

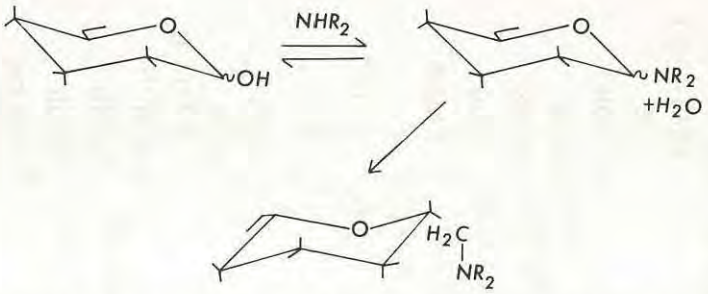
CARBONYL-AMINE REACTIONS

In the presence of ammonia, primary, or secondary amines or amino acids, the browning of reducing sugars is greatly accelerated. The initial stages of the reaction have been traced. It is known as the Maillard (cf. Ellis 1959) or the carbonyl-amine reaction (Hodge 1953), since the compounds that react with the amines usually possess a carbonyl or potential carbonyl function. The most reactive substances are simple carbonyl compounds that will caramelize even at ambient temperature; but in the presence of an amine, browning is more rapid and the color developed is intensified. Some of the most reactive carbonyl compounds are α,β-unsaturated aldehydes, such as furaldehyde, and α-dicarbonyl compounds, such as pyruvaldehyde and diacetal.

α,β-UNSATURATED ALDEHYDE α-DICARBONYL COMPOUND

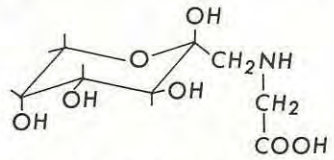
Reynolds (1970) has outlined the major features of the initial stages of the carbonyl-amine reaction based not only on her extensive

studies, but also those of Hodge (e.g., Hodge *et al.* 1963). At the outset, an aldose or ketose sugar reacts with a primary or secondary amine to form a glycosylamine, and the reaction is reversible. When glucose reacts with ammonia the initial product is glucosylamine, which rearranges in the presence of an acid catalyst to yield 1-amino-1-deoxy-D-fructose:



The role of water is important in determining the yield of glycosylamine. At low water content, there is substantial formation of this compound; therefore, carbonyl-amine browning is believed to be a significant pathway for browning in dried and concentrated foods. The probable mechanism for the formation of glycosylamine is addition of the amine to the carbonyl group of the open-chain form of the sugar, followed by elimination of a molecule of water and subsequent ring closure. The mechanism for the formation of 1-amino-1-deoxy-2-ketose is known as the Amadori rearrangement (Hodge 1955), and is analogous to the enolization and isomerization reactions shown by unsubstituted sugars (Chapter 4).

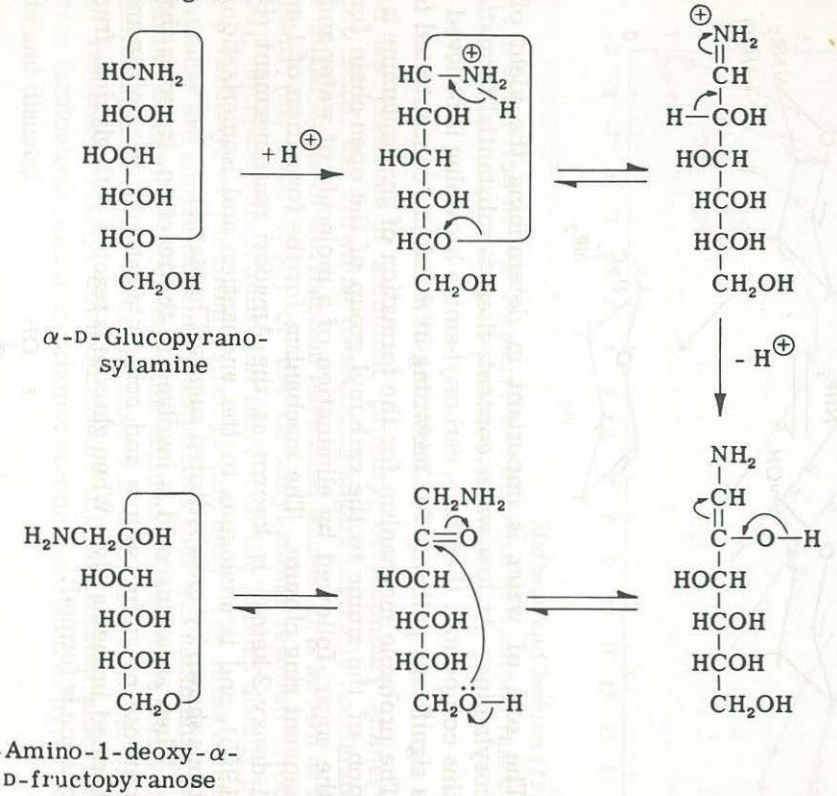
When an amino acid is involved in the reaction, it serves the dual purpose of amine source and acid catalyst, and the ketoseamine is formed immediately. When glucose is reacted with glycine, fructose-glycine is formed:



1-DEOXY-1-GLYCINO-β-D-FRUCTOSE

The ketoseamines are fairly stable, colorless compounds with greater browning reactivity than free ketoses; they are formed in low yield in dilute solutions, however, and maximum yield occurs in systems with 18% water content.

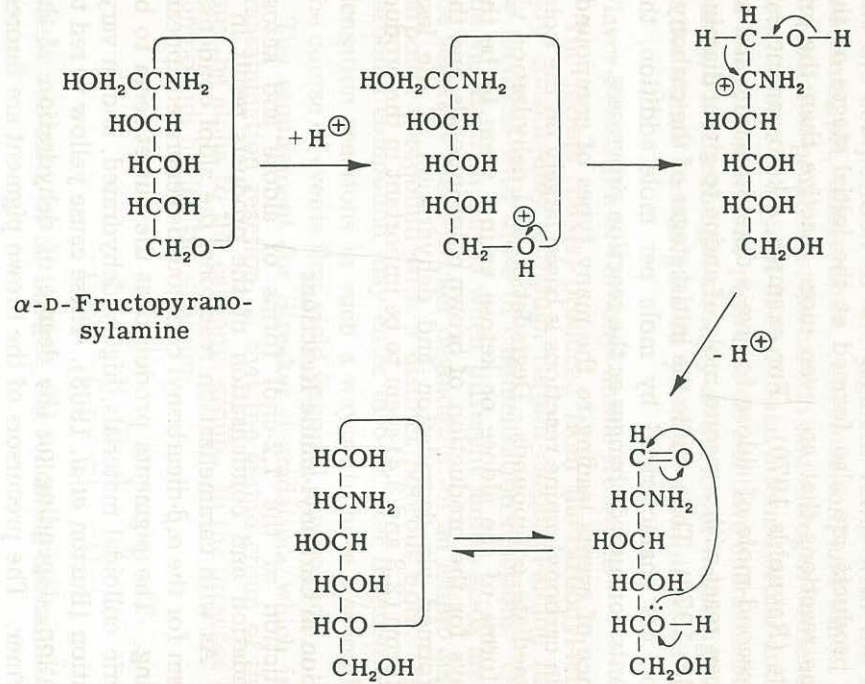
Amadori Rearrangement



α-D-Glucopyranosylamine

1-Amino-1-deoxy-α-D-fructopyranose

Heyns Rearrangement



Kort (1970)

FIG. 7.4. ACID-CATALYZED MECHANISMS FOR THE AMADORI AND HEYNS REARRANGEMENTS

Reproduced with permission of Academic Press.

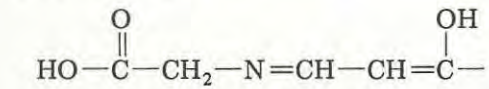
Fructose, in the presence of an amine yields initially fructosylamine which, by virtue of the Heyns rearrangement, yields glucosylamine and mannosylamine (Kort 1970). The Amadori and the Heyns rearrangements are two alternatives for the same type of transformation. The former involves protonation of the nitrogen atom at C-1, and the latter involves protonation of the oxygen atom at C-6. The rearrangements take place in either acid or basic solutions; the acid-catalyzed mechanisms are shown in Fig. 7.4.

Secondary products are also formed at the initial stages of the carbonyl-amine reactions that are even more reactive than the primary products (Reynolds 1970). For example, a ketoseamine can react with a second mole of aldose to give a diketoseamine, and an aldoseamine can react with a second mole of amine to give a diamino sugar (Reynolds 1970). Thus, while the initial stages of the carbonyl-amine reaction are characterized by mole per mole addition, the ratio of reactants consumed changes as the reaction progresses.

The sequence of events leading to the many types of compounds encountered in carbonyl-amine reactions is based largely on the isolation of intermediate compounds. Degradation and dehydration via the 1,2-enol forms of the aldose or ketose amines seem to be the major pathways for the production of brown color in foods, and the compounds formed via degradation and dehydration of the less-favored enol forms (2,3 and 3,4) seem to be important in the production of flavor.

Color Formation in Carbonyl-amine Reactions

With production of the 1,2-enol forms of aldose and ketose amines, degradation and condensation of the products result in a brown color. As with caramelization reactions, the aldol condensation mechanism for the α,β-dicarbonyl compounds generated appears to be operating. The pigments produced at the outset seem to be small polymeric colloidal materials, highly dehydrated, and in varying concentration (Burton *et al.* 1963). These cause yellow to red to brown coloration, depending on the degree of dehydration of the colloidal polymer. The precursors of the brown pigment are fluorescent, but are not identical with them. A probable structure of a fluorescent compound from a glucose-glycine browning system (Adhikari and Tappel 1973) is:



The sequential formation of the brown pigments from the carbonyl-amine reactions parallels, in many respects, the formation of

caramel pigments in caramelization reactions. For example, as the reaction between an amino acid and a sugar progresses, dark products are gradually formed; these appear one after another, as if they corresponded to successive stages of an overall reaction (Danehy and Pigman 1951). Since the carbonyl-amine pigments contain nitrogen, however, they are called "melanoidins."

Many attempts have been made to produce a standard melanoidin, but the composition of the pigment varies with the nature of the reactants, pH, temperature, concentration, and time. In general, as the reaction progresses, the carbon and nitrogen contents of the products increase as a result of continued intermolecular and intramolecular dehydration. The pigments do not have characteristic absorption bands, but absorb light strongly at the lower wavelengths, and weakly at the higher wavelengths to yield an asymptotic absorption curve.

Flavor Formation in Carbonyl-amine Reactions

The flavors (odors) produced from the carbonyl-amine reactions vary considerably, although certain carbonyl-amine flavors seem to be identical with those of caramelization reactions. One reason for the variation in flavor when different amino acids are reacted with the sugars is that Strecker degradation of an amino acid *may* yield a carbonyl compound with a distinct odorous note. Moreover, that carbonyl compound may enter into or alter fragmentation and condensation reactions in such a way as to have a secondary effect on the course of events that yield flavorful compounds.

The requirement for Strecker degradation, which is essentially a transamination reaction, is that the amino group of the amino acid must be alpha to the carbonyl group. In the presence of dicarbonyl compounds having the structure

$$-\overset{\overset{O}{\|}}{C}- \left[-\overset{\overset{H}{|}}{C}=\overset{\overset{H}{|}}{C}- \right]_n -\overset{\overset{O}{\|}}{C}-$$

where n is zero, or an integer, the following transamination reaction occurs:

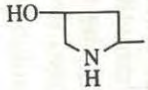
$$R-\overset{\overset{O}{\|}}{C}-\overset{\overset{O}{\|}}{C}-R + R'CHNH_2 \longrightarrow R'CHO + CO_2 + R-CHNH_2-\overset{\overset{O}{\|}}{C}-R$$

Through Strecker degradation, a new aldehyde, having one less carbon atom than the carbon atom chain of the amino acid, and also a new amine compound are generated. Examples of dicarbonyl compounds produced by the action of heat on the sugars are pyruvic acid, diacetyl, and 2-furaldehyde. The last yields an α-diketone and a con-

TABLE 7.2

FLAVORS OF HEATED 1:1 α-AMINO ACID-GLUCOSE MIXTURES

α-Amino Acid	Formula $R \cdot CH(NH_2) \cdot COOH$ R=	Volatile Strecker Aldehyde RCHO	Aroma Noted by Panel 100°C	180°C	Reference[1]
None			None	Caramel	H-S
Glycine	H—	Formaldehyde	Caramel	Burnt sugar	H-S
			Caramel		K-N-M
α-Alanine	CH_3—	Acetaldehyde	Caramel, sweet	Burnt sugar	H-S
α-Aminobutyric	$CH_3 \cdot CH_2$—	Propionic	Caramel	Burnt sugar	H-S
			Maple syrup		B-K
Valine	$(CH_3)_2CH$—	Isobutyric	Rye bread	Penetrating chocolate	H-S
			Fruity aromatic		K-N-M
Leucine	$(CH_3)_2CH \cdot CH_2$—	Isovaleric	Sweet, chocolate	Burnt cheese	H-S
			Toasted, bready		K-N-M
Isoleucine	$CH_3 \cdot CH_2 \cdot CH(CH_3)$—	2-Methyl-butanal-(1)	Musty	Burnt cheese	H-S
			Fruity aromatic		K-N-M
Serine	$HO \cdot CH_2$—	Glycolic	Maple syrup		B-K
Threonine	$CH_3 \cdot CHOH$—	Lactic	Chocolate	Burnt	H-S
Methionine	$CH_3 \cdot S \cdot CH_2 \cdot CH_2$—	Methional	Potato	Potato	H-S
Phenylglycine	C_6H_5—	Benzaldehyde	Bitter almond		B-K
Phenylalanine	$C_6H_5 \cdot CH_2$—	α-Toluic	Violets	Violets, lilac	H-S
			Rose perfume		K-N-M

Tyrosine	$HO \cdot C_6H_4 \cdot CH_2-$	Caramel		H-S
Proline		Burnt protein	Pleasant, bakery aroma	H-S
Hydroxyproline		Potato		K-N-M
Histidine		None	Cornbread	H-S
			Buttery note	K-N-M
Arginine	$H_2N \cdot C{:}NH \cdot NH \cdot (CH_2)_2-$		Burnt sugar	H-S
		Buttery note		K-N-M
Lysine·HCl	$H_2N \cdot (CH_2)_4-$	None	Bread-like	H-S
Aspartic	$HOOC \cdot CH_2-$	Rock candy	Caramel	H-S
Glutamic	$HOOC \cdot CH_2 \cdot CH_2-$	Caramel	Burnt sugar	H-S
Glutamine	$H_2N \cdot CO \cdot CH_2 \cdot CH_2-$	Chocolate, pleasant	Butterscotch	H-S
Cysteine·HCl	$HS \cdot CH_2-$	Sulfide		H-S
		Meaty		K-N-M
Cystine	$-CH_2 \cdot S \cdot S \cdot CH_2-$	Sulfide		H-S
		Burnt turkey skin		K-N-M

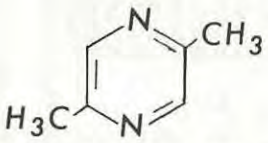
Source: Hodge (1967).

[1] B-K = Barnes and Kaufman 1947; H-S = Herz and Shallenberger 1960; K-N-M = Kiely *et al.* 1960.

jugated dicarbonyl compound upon hydrolytic opening of the ring (Hodge 1967).

The amount of aldehyde formed by Strecker degradation is probably quite small and the effect of the aldehyde on flavor is probably the result of secondary reactions (Barnes and Kaufman 1947). Amino acids that do not yield an aldehyde with characteristic flavor, or that are not particularly reactive in a browning system, can be expected to yield only characteristic caramel-like flavors. Some examples are tyrosine, glycine, and alanine. Hodge (1967) has tabulated various odors produced from carbonyl-amine reactions, some of which are shown in Table 7.2.

Pyrazine compounds with varying degrees of substitution are also generated in carbonyl-amine reactions that are severe enough to cause Strecker degradation of the amino acids. For example, Dawes and Edwards (1966) isolated 2,5-dimethylpyrazine from a glucose, glycine reaction mixture:



2,5-DIMETHYLPYRAZINE

Various alkyl pyrazine compounds are believed to be primarily responsible for the flavor of foods that undergo high-temperature processing, such as coffee, cocoa, peanuts and potato chips (Koehler and Odell 1970). There is more than one route by which different compounds form pyrazines, and the yield of pyrazines varies with different sugars. Koehler and Odell (1970) found that fructose, when reacted with asparagine, yields three times as much dimethylpyrazine as glucose. Imidazoles and pyrazines are formed by the reaction of glucose with ammonia, and Kort (1970) has proposed several mechanisms for this reaction.

FACTORS AFFECTING NONENZYMIC BROWNING REACTIONS

It is not possible to discuss the effect of any one constant in the browning reactions without mentioning, in a qualifying manner, the effects of others. This is because temperature, pH, nature and concentration of the reactants, time, and moisture content interact to such an extent that the reaction exhibits qualitative changes as it progresses. At a given temperature, for example, the reaction is accompanied by pH change, and the use of a buffer affords either the addition of a catalyst or another reactant.

Temperature

The effect of increasing temperature on any one browning system is manifested in several ways. As temperature is increased, compounds are generated that may either enter into or inhibit the reaction. Sucrose is generally inert at relatively low temperatures, but when conditions lead to its hydrolysis to glucose and fructose, these compounds readily enter into caramelization and carbonyl-amine reactions. The amino acids themselves catalyze sucrose hydrolysis in the neutral pH range, and the formaldehyde produced by the Strecker degradation of glycine can effectively block the participation of other amino acids in the reaction. It is for these reasons that accelerated temperature studies of the browning reactions can rarely be used to predict the long-term stability and browning potential of food systems at lower temperatures.

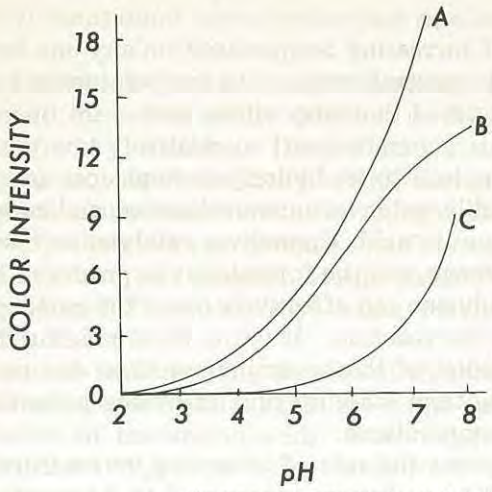
In model systems the rate of browning, as measured by color development or CO_2 evolution, increases 2 to 3 times for each 10° rise in temperature. For natural systems, such as foods with high sugar content, the rate is even more rapid and may reach the explosive range (Frantisek *et al.* 1972); the fact that fructose decomposition increases 5 to 10 times with each 10° increase in temperature has been mentioned earlier in this chapter.

As measured by amino nitrogen loss, the browning reactions appear to increase *uniformly* with rise in temperature between 0° and 90° (Lea and Hannan 1949). Other ways in which the effect of temperature is manifested are increasing carbon content in the pigment and more pigment formed per mole of CO_2 released at higher temperatures. Color intensity also increases per unit of CO_2 evolved (Ellis 1959).

Effect of pH

Changing the pH of a browning reaction system over a wide range again leads to qualitatively different reactions. This is clearly depicted by the color intensity curve for glucose in Fig. 7.2. Generally, browning reactions are slowed by decreasing pH until the pH for optimum stability of reducing sugars is passed, although this seldom happens with food products. The carbonyl-amino acid reaction is self-inhibitory in the sense that pH decreases with loss of the basic amino group.

Studies of the effect of pH on the nature of the nonenzymic browning reactions are complicated by the fact that buffer components themselves may be either browning reactants or catalysts (phosphates, phthalates, amino acids, etc.). Barnes and Kaufman (1947) found that the glucose-glycine browning rate at pH 4, as measured



Smirnov and Geispits (1956)

FIG. 7.5. DEPENDENCE OF COLOR INTENSITY OF A GLUCOSE-GLYCINE SOLUTION ON pH

A—Total color. B—Color due to non-melanoidin products. C—Color from melanoidin formation (A minus B).

by color development, doubled at pH 6; however, the glucose-glycine mixture was a 50% aqueous solution, and the effect of pH on these browning reactions is markedly influenced by moisture content.

In an attempt to distinguish between caramelization reactions and carbonyl-amine reactions Smirnov and Geispits (1956) followed glucose caramelization and glucose-glycine browning at various pH values at 100°. By subtracting the color values obtained from the caramelization phenomenon from the color values obtained from carbonyl-amine browning, they were able to allot to each system its relative contribution to color development. It would appear (Fig. 7.5) that carbonyl-amine browning does not take on much significance until the pH is greater than 6. Similar results have led others (Schroeder *et al.* 1955) to take the position that in water solution browning is primarily due to caramelization reactions, but in the nearly dry state, or at an alkaline pH, the Maillard reaction prevails. These results seem to explain why the presence of water prevents the deleterious effect of autoclaving on the nutritive value of protein.

Water and Reactant Concentration

Water catalyzes the enolization of the reducing sugars, and the enolic forms are readily fragmented and dehydrated. It follows, therefore, that moisture content should have a marked effect on the rate of browning. As mentioned previously, changing the concentra-

tion of the reactants alters the course of the reaction (Ambler 1929), and the yield of melanoidin may be increased 3 to 5 times when the concentration of reactants is only doubled. Wolfrom and Rooney (1953) found that the reaction between xylose and glycine is at a maximum at 30% moisture content, and at a minimum at moisture contents of zero and 90%. The decrease in reaction at moisture contents above 30% is undoubtedly due to dilution of the reactants. The decrease in reaction at moisture contents lower than 30% is due to the intrinsic ability of the sugars to lower water activity (Labuza *et al.* 1970). When glucose-glycine mixtures are stored under different relative humidities, browning is at a minimum at zero and 100% relative humidity, and at a maximum at 70% (Richards 1956). Under conditions of high temperature and relatively short times, the presence of water retards the Maillard reaction (Schroeder *et al.* 1955).

Nature of Reactants

Sugars.—The reactivity of the sugars in nonenzymic browning reactions generally parallels their reactivity in other respects. Pentoses are more reactive than hexoses, and hexoses in turn are more reactive than reducing disaccharides. Nonreducing disaccharides participate in browning reactions after they are first hydrolyzed to their component monosaccharides. Within a closely related series of diastereoisomers, such as the aldopentoses or the aldohexoses, reactivity is related to the conformational stability of the favored hemiacetal ring structure (Burton and McWeeny 1963). Of the naturally occurring aldopentoses, L-arabinose is more reactive than D-xylose. Among the naturally occurring aldohexoses, the order of reactivity is mannose > galactose > glucose. This is about the same order as their conformational stability, as measured by the amount of open-chain structure present in solution with Schiff's reagent, by polarographic techniques, or by instantaneous reaction with hydrogen cyanide.

The relative reactivities of the sugars in the browning reactions, may be applicable only to the particular conditions employed. Initially fructose and glycine produce more color than glucose and glycine, but the position can be reversed if the reaction is allowed to continue. Although fructose is generally considered to be quite reactive in browning systems, the loss of amino acid or free amino groups of casein is very low with fructose, as compared with glucose (McWeeny 1973).

Amino Acids.—The browning reactivity of an amino acid depends upon the method used to follow the reaction, on its concentration, and on the reactivity of the aldehyde generated when Strecker degradation occurs. Glycine and alanine are generally fairly reactive, but

at high temperatures, where Strecker degradation occurs, the investigator should take into account the possibility that the formaldehyde produced from glycine may inhibit the participation of residual glycine in the reaction due to the fact that a "formal titration" may have been inadvertently performed.

As measured by changing optical rotation, glutamic acid seems to be quite reactive, but is not necessarily so, as measured by color development. Some amino acids have more than one reactive nitrogen atom that participates in carbonyl-amine browning, but not all the constituent nitrogen of each amino acid is necessarily reactive. With the possible exception of glutamine, alpha amino groups of the amino acids readily react with an aldehyde or ketone. Presumably the amide group of glutamine reacts easily, generating pyrrolidone-carboxylic acid, an inert heterocyclic nitrogen compound in which the α-amino nitrogen atom is involved in ring closure. On the other hand, the alpha amino group of asparagine is quite reactive and the amide nitrogen atom is inert. The alpha amino nitrogen atom and one of the heterocyclic nitrogen atoms of histidine are both apparently active in high-temperature reactions, and both nitrogen atoms of lysine are reactive. However, only the alpha amino nitrogen atom of arginine is capable of reaction and as the amino group is further removed from the α-position on an aliphatic chain, it tends to become less reactive. γ-Aminobutyric acid shows little tendency to participate in carbonyl-amine reactions.

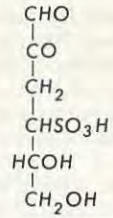
Chemical Inhibition of Browning Reactions

Although any reagent that will block the carbonyl function of the sugars minimizes browning, sulfur dioxide and sodium and potassium bisulfite represent the best means of preventing or minimizing the deteriorative effects of the browning reaction in foods (Barnes and Kaufman 1947). Sodium bisulfite can combine with the carbonyl group of an aldose to yield an aldose-bisulfite adduct (Burton *et al.* 1963):

$$NaHSO_3 + RCHO \longrightarrow RCHOHSO_3Na$$

However, such compounds are highly dissociated in solution, and at the sulfite concentrations used in foods, the sulfite could "remove" only a very small amount of the reactive sugar. At pH 4.5 the sodium and potassium bisulfite addition compounds of the sugars will crystallize from saturated solutions of aldose and bisulfite (Reynolds 1969).

At 100° at pH 6.5, a 4-sulfohexosulose has been isolated (Ingles 1962) from a browning mixture and shown to be 3,4-dideoxy-4-sulfo-D-*glycero*-hexosulose:



The 4-sulfohexosulose is presumably formed from the enolic form of 3-deoxyosulose. The formation of a chelated di-adduct has also been proposed to explain the relative stability of such compounds. This rationale explains the loss of sulfites in stored food products without sulfate, volatile sulfur, or free sulfur formation (Burton *et al.* 1963).

BIBLIOGRAPHY

ADHIKARI, H. R., and TAPPEL, A. L. 1973. Fluorescent products in a glucose-glycine browning reaction. J. Food Sci. *38*, 486–488.

AMBLER, J. A. 1929. The reaction between amino acids and glucose. Ind. Eng. Chem. *21*, 47–50.

BARNES, H. M., and KAUFMAN, C. W. 1947. Industrial aspects of the browning reaction. Ind. Eng. Chem. *39*, 1167–1170.

BURTON, H. S., and McWEENY, D. J. 1963. Nonenzymic browning reactions: Consideration of sugar stability. Nature *197*, 266–268.

BURTON, H. S., McWEENY, D. J., and BILTCLIFFE, D. O. 1963. Nonenzymic browning. Development of chromophores in the glucose-glycine and sucrose-glycine systems. J. Food Sci. *28*, 631–639.

CUNNINGHAM, M., and DORÉE, C. 1917. Contributions to the chemistry of caramel. Part I. Caramelan. J. Chem. Soc. *111*, 589–607.

DANEHY, J. P., and PIGMAN, W. W. 1951. Reactions between sugars and nitrogen compounds and their relation to certain food problems. Adv. in Food Res. *3*, 241–290.

DAWES, I. W., and EDWARDS, R. A. 1966. Methyl-substituted pyrazines as volatile reaction products of heated aqueous aldose-amino acid mixtures. Chem. and Ind. (London) 2033.

DOSS, K. S. G., and GHOSH, S. K. 1949. Kinetics of color development in invert sugar solutions. Proc. Sugar Technol. Assoc. (India) *18*, Part I. 26–29.

ELLIS, G. P. 1959. The Maillard reaction. Advances in Carbohydrate Chem. *14*, 63–133.

FRANTISEK, J., MILOSLAV, S., and KAREL, C. 1972. Explosion of an ion-exchange resin decolorizing filter. Listy Cukrov. *88*, 81–84.

GREENSHIELDS, R. N., HUNT, P. C., FEASEY, R., and MacGILLIVRAY, A. W. 1969. J. Inst. Brew. (London) *75*, 542–550.

HERZ, W. J., and SHALLENBERGER, R. S. 1960. Some aromas produced by simple sugar-amino acid reactions. Food Res. *25*, 491–494.

HODGE, J. E. 1953. Chemistry of browning reactions in model systems. J. Agr. Food Chem. *1*, 928–943.

HODGE, J. E. 1955. The Amadori rearrangement. Advances in Carbohydrate Chem. *10*, 169–205.

HODGE, J. E. 1967. Origin of flavor in foods. Nonenzymic browning reactions. *In* Chemistry and Physiology of Flavors, H. W. Schultz *et al.* (Editors). Avi Publishing Co., Westport, Conn.

HODGE, J. E., FISHER, B. E., and NELSON, E. C. 1963. Dicarbonyls, reduc-

tones, and heterocycles produced by reactions of reducing sugars with secondary amine salts. Proc. Am. Soc. Brewing Chemists, 84-92.
INGLES, D. L. 1962. The formation of sulfonic acids from the reactions of reducing sugars with sulfite. Aust. J. Chem. *15*, 342-349.
JANACEK, O. 1939. Behavior of sucrose on heating and composition of caramel substances so obtained. Centr. Zukerind. *47*, 647-648.
JURCH, JR., G. R., and TATUM, J. H. 1970. Degradation of D-glucose with acetic acid and methyl amine. Carbohyd. Res. *15*, 233-239.
KIELY, P. J., NOWLIN, A. C., and MORIARTY, J. H. 1960. Bread aromatics from browning systems. Cereal Sci. Today *5*, 273-274.
KOEHLER, P. E., and ODELL, G. V. 1970. Factors affecting the formation of pyrazine compounds in sugar-amine reactions. J. Agr. Food Chem. *18*, 895-898.
KORT, M. J. 1970. Reactions of free sugars with aqueous ammonia. Adv. in Carbohydrate Chem. and Biochem. *25*, 311-349.
KRÖNER, W., and KOTHE, H. 1939. Discoloration of dextrose solutions. Ind. Eng. Chem. *31*, 248.
LABUZA, T. P., TANNENBAUM, S. R., and KAREL, M. 1970. Water content and stability of low-moisture and intermediate-moisture foods. Food Technol. *24*, 543-550.
LEA, C. H., and HANNAN, R. S. 1949. Studies on the reaction between proteins and reducing sugars in the dry state. I. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. Biochem. Biophys. Acta *3*, 313-325.
LEDON, A. C., and LANANETA, P. 1950. Spectrophotometric comparison of the factors that affect the formation of colored substances during manufacture of sugar. Bol. ofic. asoc. téchnicos azucar. (Cuba) *8*, 547-566.
LINNER, R. T. 1973. Personal communication.
MATHEWS, J. A., and JACKSON, R. F. 1933. The stability of levulose in aqueous solutions of varying pH. J. Res. Nat. Bur. Stds. *11*, 619-633.
McDONALD, E. J. 1950. Stability of dextrose solutions of varying pH. J. Res. Nat. Bur. Stds. *45*, 200-203.
McWEENY, D. J. 1973. Carbohydrates in nonenzymic browning. *In* Molecular Structure and Function of Food Carbohydrate, G. G. Birch *et al.* (Editors). Applied Science Publishers, London.
MIROSHNIKOVA, Z. P., CHIKIN, G. A., and TOBLINA, N. M. 1970. Chemical nature and the products of sucrose caramelization. Sakh. Prom. *44*, 10-15.
O'COLLA, P. S., LEE, E. E., and McGRATH, D. 1962. The action of cation exchange resins on D-glucose. J. Chem. Soc. 2730-2733.
PICTET, A., and REILLY, J. 1921. On Levulosan. Helv. Chim. Acta *4*, 613-621.
PICTET, A., and STRICKER, P. 1924. Constitution and synthesis of isosaccharosan. Helv. Chim. Acta *7*, 708-713.
PLUNGUIAN, M., and HIBBERT, H. 1935. Studies on lignin and related compounds. XI. The nature of lignite humic acid and of the so-called "humic acid" from sucrose. J. Am. Chem. Soc. *57*, 528-536.
REYNOLDS, T. M. 1969. Nonenzymic browning. Sugar-amine interactions. *In* Carbohydrates and Their Roles, H. W. Schultz *et al.* (Editors). Avi Publishing Co., Westport, Conn.
REYNOLDS, T. M. 1970. Flavors from nonenzymic browning reactions. Food Technol. Australia. 610-619.
RICHARDS, E. L. 1956. Nonenzymic browning: the reaction between D-glucose and glycine in the dry state. Biochem. J. *64*, 639-644.
SAPRONOV, A. R., KHARIN, S. E., and PARSHINA, T. A. 1969. Adsorption of coloring materials on activated carbon in relation to their extent of dispersion. Sakh. Prom. *43*, 24-28.

SATTLER, L., and ZERBAN, F. W. 1945. Unfermentable reducing substances in molasses. Ind. Eng. Chem. *37*, 1133.

SCHROEDER, L. J., IACOBELLIS, M., and SMITH, A. H. 1955. The influence of water and pH on the reaction between amino compounds and carbohydrates. J. Biol. Chem. *212*, 973–983.

SHALLENBERGER, R. S., and PEDERSON, C. S. 1961. Stackburn. Food Technol. Australia, 211–213.

SHUMAKER, J. B., and BUCHANAN, J. H. 1932. A study of caramel color. Iowa State J. Sci. *6*, 367–379.

SMIRNOV, V. A., and GEISPITS, K. A. 1956. The formation of melanoidins. Biokhimiya *21*, 633–635.

WOLFROM, M. L., and ROONEY, C. S. 1953. Chemical interactions of amino compounds and sugar. VIII. Influence of water. J. Am. Chem. Soc. *75*, 5435–5436.

Enzymic Hydrolysis of Oligosaccharides, and Fermentation

ENZYMIC HYDROLYSIS OF OLIGOSACCHARIDES

Only four enzyme classes (Pigman 1944) have significance in the hydrolysis of the majority of the naturally occurring oligosaccharides. These classes, along with the trivial names and some substrates for the enzymes, are shown in Table 8.1. The classes of enzymes derive their names from the geometry of the glycosidic linkage and are also usually pyranosidases, though the β-fructosidases are furanosidases.

α-Glucosidases

Although the amylases of barley and yeast (β-amylases) that act on starch are α-D-glucosidases, the products of their action are the maltose, maltotriose, maltotetrose, etc. series of oligosaccharides. The term β-amylase tends to be misleading. These amylases are exo-enzymes that hydrolyze starch from the *nonreducing* end of the molecule. The principal initial product *seems to be* β-D-maltose, as indicated by the direction of mutarotation, in spite of the fact that an α-D-glucosidic bond is severed. The phenomenon was discovered by Ohlssen (1922).

The usual interpretation of the events shown by β-amylases (Reed 1966) is that they do not imply the presence of β-D-linkages in the starch molecule, but rather indicate the inversion of a configuration that may take place during enzymic reaction, since an asymmetric carbon atom is involved (i.e., a Walden inversion occurs).

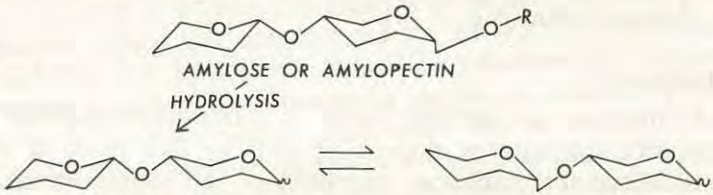
In the light of present stereochemical (conformational) principles a more likely explanation for the "upward" mutarotation of maltose,

TABLE 8.1

ENZYME CLASSES, COMMON (TRIVIAL) ENZYME NAMES AND SOME SUBSTRATES FOR THEIR ACTION

Enzyme Class	Common Name	Substrate(s)
α-Glucosidases	Maltase, trehalase	Maltose, trehalose, α-D-glucosides
β-Fructosidases	Sucrase, saccharase, invertase	Sucrose, raffinose, stachyose, β-D-fructofuranosides
α-Galactosidases	Melibiase	Melibiose, manninotriose, α-D-galactosides, β-L-arabinosides
β-Galactosides	Lactases	Lactose, β-D-galactosides, α-L-arabinosides

immediately upon being released from starch (amylose of amylopectin), is that the glucopyranose units of maltose show conformational inversion or partial inversion. It is possible that the terminal glucose unit of amylose and amylopectin has the alternate (1C) conformation, since the C1 conformation places an extremely bulky substituent (the entire starch molecule) at the anomeric center. Hydrolysis would greatly decrease the size of this substituent, with the result that maltose would establish an equilibrium between two conformers that have higher overall specific rotation as compared to the maltose conformer released immediately on hydrolysis.



Evidence for this possibility is found in the discovery (Bentley 1952) that while the reducing glucose unit of maltose seems to have the C1 conformation, the nonreducing moiety has the skew conformation intermediate between the boat conformations B1 and B3. Cellobiose has the C1 conformation for both glucose units which is to be expected of an equatorial-equatorial glycosidic linkage.

The scientific name for β-amylase (E.C. 3.2.1.2) is a α-1,4-glucan maltohydrolase indicating the type of reaction (hydrolysis), the nature of the substrate, and the end products, glucan and maltose.

α-Amylases (E.C. 3.2.1.1) are so named to distinguish them from β-amylases. Their principal action is random attack on starch molecules which ultimately converts them to maltose and glucose. This conversion is exceedingly slow, however, and is therefore the rate-limiting step (Aspinall 1970). Salivary amylase is an example of an α-amylase.

Some glucoamylases, (E.C. 3.2.1.3) especially those from *Aspergillus niger* and *Asperigillus oryzae*, slowly hydrolyze maltose to glucose (Pazur and Kleppe 1962; Maher 1969). The enzyme is also active on α, 1 $\rightarrow$ 3 (nigerose), α, 1 $\rightarrow$ 6 (isomaltose), and even β, 1 $\rightarrow$ 6 (gentiobiose) linkages as well as on sucrose, but activity is greatly reduced.

Eggermont (1969) found that the truly intestinal α-glucosidases that are bound to intestinal cellular structures are of 3 types: one is a glucoamylase that converts glycogen to glucose; the second is a maltase-isomaltase, wherein the isomaltase activity is 7 times greater than the maltase activity; the third is a maltase-sucrase, and action on

sucrose is 2.4 times as rapid as on maltose. Cattle liver possesses a lysosomal acid α-glucosidase that is able to hydrolyze glycogen to glucose by acting as an exoglucoamylase; however, glucose competitively inhibits the maltase activity of the enzyme (Sica *et al.* 1971).

As mentioned above, certain α-glucosidases can hydrolyze sucrose, and there is disagreement as to whether or not these α-glucosidases are identical with maltase. Such glucosidoinvertases are not active on the raffinose family of oligosaccharides; taka-invertase from *Aspergillus niger* is an example. Mammalian invertase (maltase?) is also an α-glucosidase apparently without action on the raffinose family of oligosaccharides.

β-Fructosidases

Yeast invertase is predominately a β-fructofuranosidase (E.C. 3.2.1.26) that hydrolyzes sucrose to glucose and fructose (invert sugar), raffinose and stachyose to melibiose and manninotriose, plus glucose respectively, and gentianose to gentiobiose plus glucose. The specificity of yeast invertase, free of α-glucosidases, for β-D-fructofuranosides was of paramount importance in establishing the structure of sucrose (Levi and Purves 1949). On the other hand, the enzyme seems to exhibit no specificity for the "afructone" portion of a molecule (Gottschalk 1950). However, the rate at which β-D-fructofuranosidase hydrolyzes the sucrose portion of the raffinose family of oligosaccharides is inversely proportional to the molecular weight. Adams *et al.* (1943) found that the ease of hydrolysis of sucrose, raffinose, and stachyose is of the order of 100 to 23 to 6.8 for baker's (ale) yeast, and 100 to 12.5 to 3.1 for brewer's yeast, in spite of the fact that brewer's yeast also contains an α-galactosidase which hydrolyzes the melibiose and manninotriose portions of raffinose and stachyose.

α-Galactosidases

The literature on α-galactosidases (E.C. 3.2.1.22) is relatively sparse, since this is not a widely occurring enzyme. Although it is found in brewer's yeast, almond emulsin, and various fungi and bacteria, it is absent from the intestines of the cow and man. The common name for α-galactosidase is melibiase, since the sugars raffinose and stachyose contain a melibiose unit. Stachyose also contains a manninotriose and galactobiose unit which serve as substrates.

Sources of α-D-galactosidase activity are of current interest to food technologists, since the occurrence of raffinose and stachyose

in foods of legume origin is not considered to be of any particular nutritional advantage. It is believed that one reason why the fermentation of soybeans in the production of the Indonesian food tempeh enhances nutritional value is that stachyose is almost completely hydrolyzed to galactose, glucose, and fructose (Shallenberger *et al.* 1966) by a rhizopus mold species. Mital *et al.* (1973) found that certain lactobacilli can hydrolyze stachyose and that the α-galactosidase is either constitutive or, as with the bacterium *Escherichia coli*, can be induced.

β-Galactosidases

The only abundant β-D-galactoside is the mammalian milk sugar, lactose; hence the common name for the β-D-galactosidase (E.C. 3.2.1.23) that hydrolyzes lactose to galactose and glucose, approximately doubling the sweetening power, is lactase. The enzyme is extractable from fungal (*Aspergillus oryzae*), yeast (*Sacchromyces fragilis*), and bacterial (*Escherichia coli*) sources (Pomeranz 1964). It is also found in seeds of alfalfa and coffee (Wallenfels and Malhotra 1961), in human saliva, and in the intestines of various mammalian species.

Upon being hydrolyzed to simple sugars, the oligosaccharides exhibit the properties of sugar mixtures, and can be fermented.

FERMENTATION

Although Pasteur defined fermentation as life without oxygen, more modern concepts are confined to a narrower range of metabolic events. Stanier *et al.* (1971) define fermentation as "an energy-yielding metabolic process in which organic compounds serve both as electron donors and electron acceptors." This definition conveniently encompasses the metabolic events associated with phosphorylated intermediates as well as the intrinsic difference between fermentation and anaerobic respiration. The major and indeed often the sole contribution of energy within a fermentation process is the provision of adenosine triphosphate (ATP), which is formed by the transfer to adenosine diphosphate (ADP) of energy-rich phosphate groups from phosphorylated intermediates that arise in the course of substrate breakdown.

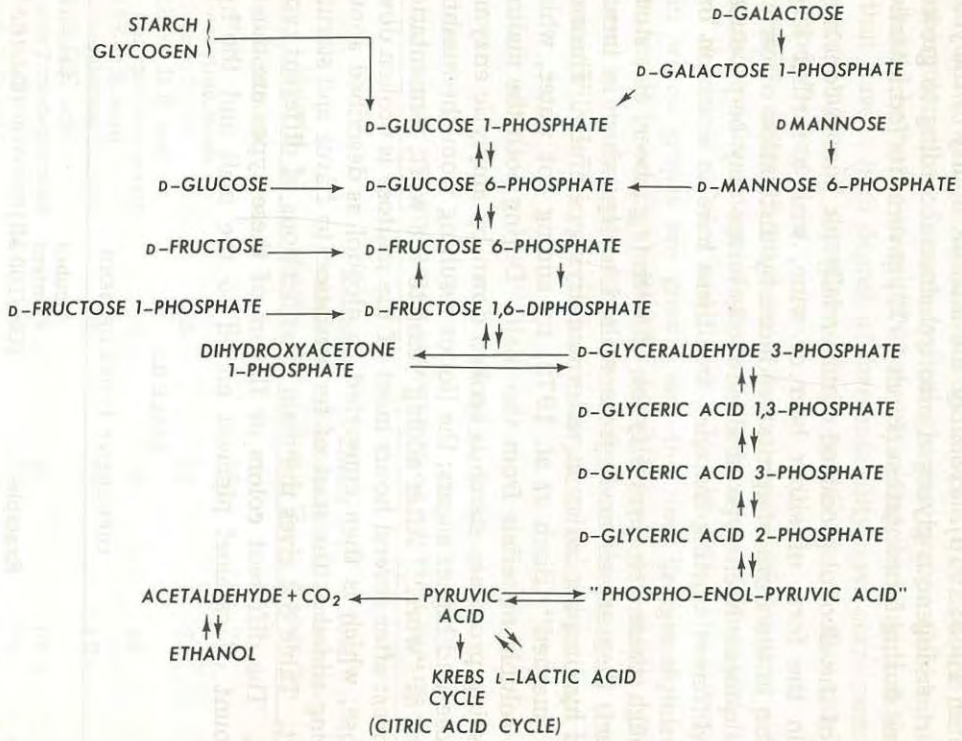
Respiration, on the other hand, by definition implies coupling of the substrate with some external source of electron acceptor that is characteristic of respiratory metabolism, and usually permits a complete oxidation of organic substrates to carbon dioxide. The free energy change for complete oxidation of an organic compound is, therefore, very much greater than for its fermentation. For example,

complete oxidation of 1 mole of glucose liberates 688 Calories, whereas most fermentations of this sugar liberate about one-tenth as much energy.

Alcoholic Fermentation

The ability of homogenized cell-free yeast juice to ferment sucrose was accidently discovered by the Buchners in 1897 (Baldwin 1953), and this was the key to the multi-stage biochemical process of fermentation as it is known today. Yeast juice contains all the enzymes present in the organism that catalyze each stage of the process. Fermentation is accomplished by various strains of *Saccharomyces* selected for their ability to yield products of consistent quality in a reproducible way. Although yeasts normally only ferment a few sugars, they can be adapted to ferment others; in particular they can ferment oligosaccharides by development of the specific hydrolytic enzymes necessary to convert them to monosaccharides. Ordinary yeasts ferment glucose at alkalinities up to pH 8; but maltose is fermented only slowly above pH 7.2 (Noggle 1957), and dextrins above this molecular weight are hardly affected. These differences may be harnessed to advantage in the analysis of the complex mixtures of sugars encountered in the brewing industry. Thus the determination of the reducing power of a sample before fermentation, after fermentation at pH 7.5 and after fermentation at pH 5.0, provides a selective method for the estimation of glucose, maltose and unfermentable dextrins in a complex mixture. The enzymes of fermentation are the same as those involved in the Embden-Myerhof-Parnas (EMP) scheme of glycolysis, which may be considered to begin with glucose or fructose (from sucrose), or with the polysaccharides starch or glycogen, and end with the production of either ethanol or lactic acid as shown in Fig. 8.1.

Monosaccharides such as fructose, galactose, and mannose are isomerized after phosphorylation to glucose 1-phosphate, and hence pass through the same metabolic sequence of events (Noggle 1957). At each phosphorylation stage 1 mole of ATP is used up and converted to ADP, while at each dephosphorylation stage the opposite reaction occurs, so that overall 2 new moles of ATP are produced for every mole of glucose oxidized. This is the immediate source of energy for the fermentation process. Glycolysis and fermentation are anaerobic processes. If oxygen is present, ethanol and lactic acid are not formed, and the pyruvic acid of the glycolytic pathway is converted further to oxidation products via the Krebs tricarboxylic acid cycle with production of many more moles of ATP.



Noggle (1957)

FIG. 8.1. EMBDEN-MEYERHOF-PARNAS SCHEME OF GLYCOLYSIS

Reproduced with permission of Academic Press.

The capacity of any yeast to carry out the fermentative process depends on its quality as well as its strain; in a dried yeast preparation only a very small proportion of all the cells may be viable. The yeast cells themselves contain the 3 polysaccharides, glycogen, glucan and mannan, as well as the key energy reserve disaccharide α,α-trehalose, which is found in a fermenting mixture in phosphorylated form (Birch 1963; 1970). Probably the intrinsic ability of the yeast to convert endogenous glycogen into trehalose according to growth conditions during fermentation (Birch 1973) governs its fermentative quality.

Most of the alcohol produced commercially for human consumption is in the form of either beer or wine, with a further large proportion in the form of spirits produced by distillation of certain brewed liquors. Each type of alcoholic beverage may be produced from a different starting material, but these have in common one or more simple sugars.

Although there are several types of beer (e.g., beer, ale, stout, and lager), they are all made from malted barley, which in turn is obtained by steeping barley in water and drying carefully. The malt is then "mashed" (Birch *et al.* 1972) by adding hot water, which extracts soluble materials from the malt. During both the malting and mashing processes starch is broken down by amylytic enzymes to maltose and higher sugars; the liquor resulting from the mashing is known as "wort." After adding yeast to the wort, fermentation commences; after several hours most of the maltose is broken down to glucose, which is then converted to alcohol as described above, hops being added at the start of fermentation to flavor and sterilize the wort. Table 8.2 gives the main classifications of different types of beer. The different colors or flavors of these types are due to the amount of caramel pigment added to the malt and the hop

TABLE 8.2

DIFFERENT TYPES OF BEER

Type	Example	Alcohol Content (Gm/100 Ml)	Calorific Value (Cal/100 Ml)
Light beers	Bitter	3.1	31
	Pale ale	3.3	32
Dark beers	Brown ale	2.2	28
	Mild ale	2.6	25
	Stout	2.9	37
Strong beers	Stout, extra	4.3	39
	Strong ale	6.6	73

extractives. In beer production the brewer is dependent on natural diastase in the barley for the formation of sufficient maltose from starch to make the alcoholic fermentation economic as a commercial process. Inevitably control is somewhat limited because of fluctuations in barley quality. Modern brewers can achieve greater flexibility and control in beer production by using glucose syrups, instead of or as well as standard wort. Table 8.3 lists the fermentation characteristics of some typical glucose syrups (Maiden 1970). It should be borne in mind when consulting the table that those syrups that have a high degree of fermentability have correspondingly low percentages of dextrins, which contribute to the thickness and "body" of the beer.

Wine differs from beer in being an entirely natural product prepared by the action of yeasts on the natural fermentable substances of the grape. Color and flavor vary with the grape variety, the area in which grapes are grown, and the time they have been allowed to stand in contact with the skins during fermentation. If fermentation is continued until all the sugars are consumed, the resulting wine will be "dry." However, as yeasts cannot tolerate an alcohol

TABLE 8.3

FERMENTATION PROPERTIES OF GLUCOSE SYRUPS

Material	Readily Fermentable Sugars, %	Slowly Fermentable Sugars, %	Total Fermentable Sugars, %	Non-fermentable Carbohydrates, %
Infusion mash wort	60	15	75	25
Decoction mash wort	65	12	77	23
18.5% D.E. acid-enzyme conversion	10	5	15	85
42% D.E. acid conversion	33	12	45	55
42% D.E. acid-enzyme conversion	47.5	15	62.5	37.5
55% acid conversion	50	15	65	35
63% D.E. acid-enzyme conversion	71	8	79	21
72% D.E. acid-enzyme conversion	69	8	77	23
93% D.E. acid-enzyme conversion	78	10	88	12
100% D.E. (dextrose)	88	5	93	7
	100	0	100	0

Source: Maiden (1970).

TABLE 8.4

FERMENTATION CHARACTERISTICS OF SOME MICROORGANISMS

Organism	Sorbitol	D-Gluconic Acid	Dulcitol	D-Galactonic Acid	Mucic Acid	Inositol	Erythritol	Methyl α-D-glucoside	Glucosamine	D-Gluconononose
Bacillus megatherium	0	A	0	0	0	0	0	0	0	0
Serratia marcescens	A	A	0	0	0	A	A	0	A	0
Escherichia coli	AG	AG	AG	AG	A	0	0	0	AG	0
Acrobacter aerogenes	AG	AG	0	0	AG	AG	0	AG	AG	0
Bacterium friedländeri	AG	AG	AG	AG	AG	AG	0	AG	AG	0
Proteus vulgaris	0	AG	0	0	0	0	0	AG	0	A
Salmonella aertrycke	AG	AG	AG	AG	AG	0	0	0	A	0
S. enteritidis	AG	A	AG	A	A	0	0	0	A	0
S. choleraesuis	AG	AG	0		0	0	0	0	A	0
S. paratyphi	AG	AG	A	0	0	0	0	0	A	0
S. schottmülleri	AG	AG	AG	AG	A	AG	0	0	A	0
Eberthella typhi	A	A	0	0	0	0	0	0	0	0
E. dysenteriae, Flexner	A	A	0	A	0	0	0	A	0	0
E. dysenteriae, Sonne	0	A	0	A	A	0	0	0	0	0
Sarcina lutea	0	0	0	0	0	0	0	0	0	0
Staphylococcus aureus	0	A	0	0	0	0	0	0	A	0
Staph. albus	A	A	A	0	A	A	A	A	A	A
Saccharomyces cerevisiae	0	0	0	0	0	0	0	A	0	0
Torula cremoris	0	0	0	0	0	0	0	0	A	A

Source: Noggle (1957).

A, acid formation observed.
G, gas formation observed.
0, No reaction.

TABLE 8.5

FERMENTATION CHARACTERISTICS OF SOME MICROORGANISMS

Organism	D,L-Glyceric aldehyde	D-Arab-inose	L-Arab-inose	D-Ribose	L-Ribose	D-Lyxose	D-Xylose	L-Rham-nose	D-Glucose	D-Man-nose	D-Galac-tose
Bacillus megatherium	A	0	A	A	0	0	A	0	A	AG	0
Serratia marcescens	AG	A	A	A	0	A	0	0	A	A	A
Escherichia coli	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Aerobacter aerogenes	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Bacterium friedländeri	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Proteus vulgaris	A	A	0	0	A	A	A	0	A	A	0
Salmonella aertrycke	AG	A	AG	AG	A	AG	AG	AG	AG	AG	AG
S. enteritidis	A	A	AG	AG	A	A	AG	AG	AG	AG	A
S. Choleraesuis	A	A	0	AG	0	AG	AG	AG	AG	AG	AG
S. paratyphi	A	0	AG	AG	A	A	A	AG	AG	AG	AG
S. schottmülleri	A	A	AG	AG	AG	AG	AG	AG	AG	AG	AG
Eberthella typhi	A	0	0	A	0	0	0	0	A	A	0
E. dysenteriae Flexner	A	0	A	A	A	0	0	0	A	A	0
E. dysenteriae Sonne	A	A	A	A	A	A	A	A	A	A	A
Sarcina lutea	A	0	0	0	0	A	0	0	0	0	0
Staphylococcus aureus	A	0	A	A	A	0	A	0	A	0	0
Staph. albus	A	0	A	A	A	A	A	0	A	A	A
Saccharomyces cerevisiae	0	0	0	0	0	0	0	0	AG	AG	0
Torula cremoris	0	0	0	0	0	0	0	0	AG	AG	0

Source: Noggle (1957).

A, acid formation observed.
G, gas formation observed.
0, no reaction.

content greater than about 16%, natural (unfortified) wines cannot have an alcohol content greater than this. In general they contain about 10% alcohol.

Malo-lactic Fermentation

It has been known since about the turn of the century that, after alcoholic fermentation by yeasts, certain lactic acid bacteria may carry out a secondary fermentation of wine. During this secondary fermentation the dicarboxylic malic acid is converted into the monocarboxylic lactic acid, thus resulting in considerable de-acidification with concurrent gas (CO_2) production (Kunkee 1967). This may be desirable in wines of high acidity, but detrimental to wines that are already low in acids. In addition to the de-acidification, further flavor changes may take place, either favorable or unfavorable, and it is therefore clearly advantageous to be able to control the process commercially. Unfortunately malo-lactic bacteria are difficult to control under such conditions, but they can be cultivated with the aid of a growth factor present in tomato juice. Some interesting recent work has shown that this growth factor is also a carbohydrate (Amachi *et al.* 1971), and is in fact the 4′-*O*-β-D-glucoside of D-pantothenic acid.

Acetic Acid Fermentation

Alcoholic fermentation of malt, followed by acetous fermentation, gives rise to vinegar, which is an aqueous solution containing about 5% acetic acid and other minor components from the malt. It is the presence of these minor products that allows vinegar to be distinguished from artificial vinegar or the non-brewed condiment obtained from pure acetic acid and water.

Fermentation Characteristics of Microorganisms

Microorganisms may be classified according to whether they produce acid or gas, or both acid and gas, when allowed to ferment specific sugars. In this way the organisms may be identified and guarded against in foods, where they may cause canned materials to spoil and burst their containers. However, most food spoilage organisms do not, fortunately, cause food poisoning. A list of some typical microorganisms and their effects on various sugars (Noggle 1957) is given in Tables 8.4 and 8.5.

BIBLIOGRAPHY

ADAMS, M., RICHTMYER, N. K., and HUDSON, C. S. 1943. Some enzymes present in highly purified invertase preparations; a contribution to the study

of fructofuranosidases, glucosidases, galactosidases and mannosidases. J. Am. Chem. Soc. *65*, 1369-1380.

AMACHI, T., IMAMOTO, S., and YOSHIZUMI, H. 1971. A growth factor for malo-lactic fermentation bacteria. Part II. Structure and synthesis of a novel pantothenic acid derivative isolated from tomato juice. Agr. Biol. Chem. *35*, 1222-1230.

ASPINALL, G. O. 1970. Polysaccharides. Pergamon Press, Oxford.

BALDWIN, E. 1953. Dynamic Aspects of Biochemistry, Cambridge University Press.

BENTLEY, R. 1959. The conformations of the disaccharides cellobiose and maltose. J. Am. Chem. Soc. *81*, 1952-1956.

BIRCH, G. G. 1963. Trehaloses. Advances in Carbohydrate Chem. *18*, 201-225.

BIRCH, G. G. 1970. Mushroom sugar in food technology. Process Biochem. *5*, 9.

BIRCH, G. G. 1973. Mushroom sugar. Plant Foods *1*, 49-55.

EGGERMONT, E. 1969. Hydrolysis of the naturally occurring α-glucosides by the human intestinal mucosa. Eur. J. Biochem. *9*, 483-487.

GOTTSCHALK, A. 1950. Principles underlying enzyme specificity in the domain of carbohydrates. Advances in Carbohydrate Chem. *5*, 49-78.

KUNKEE, R. E. 1967. Malo-lactic fermentation. Advances in Applied Microbiol. *9*, 235-279.

LEVI, I., and PURVES, C. B. 1949. The structure and configuration of sucrose. Advances in Carbohydrate Chem. *4*, 1-35.

MAHER, G. G. 1969. Action of *Aspergillus niger* glucosidases on 1 → 6 linked glucose disaccharides. Staerke *21*, 66-67.

MAIDEN, A. M. 1970. Food and fermentation applications of starch hydrolyzates. *In* Glucose Syrups, G. G. Birch *et al.* (Editors). Elsevier Publishing Co., Amsterdam.

MITAL, B. K., SHALLENBERGER, R. S., and STEINKRAUS, K. H. 1973. α-D-Galactosidase activity of *Lactobacilli*. Applied Microbiol. *26*, 783-788.

NOGGLE, G. R. 1957. Identification and quantitative determination of carbohydrates. Also, Photosynthesis and metabolism of carbohydrates. *In* The Carbohydrates. W. W. Pigman (Editor), Academic Press, Inc., New York.

OHLSSEN, E. 1922. On the existence of two amylases in the diastase of malt. Compt. Rend. Soc. Biol. *87*, 1183-1184.

PAZUR, J. H., and KLEPPE, K. 1962. The hydrolysis of α-D-glucosides by amyloglucosidase from *Aspergillus niger*. J. Biol. Chem. *237*, 1002-1006.

PIGMAN, W. W. 1944. Specificity, classification, and mechanism of action of the glycosidases. Advances in Enzymology *4*, 41-74.

POMERANZ, Y. 1964. Lactase (β-D-galactosidase). I. Occurrence and properties. Food Technol. *18*, 682-687.

REED, G. 1966. Enzymes in Food Processing. Academic Press, New York.

SHALLENBERGER, R. S., HAND, D. B., and STEINKRAUS, K. H. 1966. Changes in sucrose, raffinose, and stachyose during tempeh fermentation. 8th Dry Bean Research Conference, Agricultural Research Service, USDA Publication ARS-74-41, 68-71.

SICA, V., SIANI, A., BRUNI, C. B., and AURICCHIO, F. 1971. Maltase, isomaltase and glucoamylase activities of the acid α-D-glucoside glucohydrolase isolated from cattle liver. Biochem. Biophys. Acta *242*, 422-427.

STANIER, R. Y., DOUDOROFF, M., and ADELBERG, E. A. 1971. General Microbiology. Macmillan, London.

WALLENFELS, K., and MALHOTRA, O. M. 1961. Galactosidases. Advances in Carbohydrate Chem. *16*, 239-298.

Index

Absorption bands, hydroxyl groups, 59
α- and β-sugars, 59
Absorption of sugars, intestinal, 128
active, 129–131
spectra, 58
Acetal formation, 83–85
Acetals, acid and alkali stability, 84
Acetic acid fermentation, 204
Acetylation, 81
Acetylformoin, in caramel flavor, 171
Acids, action on sugars, 74–76
taste, 154
Acrose, 104
Addition compounds, cyanohydrin, 79
Adenosine, 142
Adenosine diphosphate, 143, 197
Adenosine triphosphate, 143–144, 197
Adenylic acid, 142
ADP. *See* Adenosine diphosphate
Agar, 47
Aglycogeusia, 127
Aglycone, 46
AH,B theory of sweet taste, 114
common unit for sweet taste, 154
Alcohol, primary groups, blocking of, 83
reactivity, 83
role in intestinal absorption, 130
secondary groups, modification, 83
solubility of sugars, 60
Alcoholic fermentation, 198
Aldaric acids, 79
Aldehydo sugars, concentration in solution, 93
Aldohexofuranose conformations, 37
Aldohexopyranose conformations, 33
1,6-anhydro-β-D-, 106
Aldohexoses, anhydride formation, 106
family tree, 22
natural occurrence, 47
Aldol condensation, 173, 182
Aldonic acids, 77–78, 80
to distinguish between aldoses and ketoses, 78
Aldopentofuranose conformations, 37
Aldopentopyranose conformations, 33
Aldose amines, 182
Aldoses, complex mutarotation configurations, 92
conformational isomers, 29
conversion to ketoses, 80
definition, 7
oxidation to aldonic acids, 77
phenylhydrazone formation, 72
reaction with hydroxylamine, 72
Aldulose, deoxy, 105
Alginates, 47
Alkali ion inhibition of sugar transport, 130
Alkalies, action on sugars, 76–77
action on sugar ethers, acetals and glycosides, 76
Alkaline phosphatase, 132
Allonic acid, 80
Allose, D-, aldehydo structure, 22
chair conformation, 33
preparation from ribose, 80
Allotropic crystalline sugars, 62
Allulose, D-, 40
chair conformation, 40
Alternate chair conformations, 13
Altroheptulose, D-, 39
Altronic acid, 80
Altrose, D-, aldehydo structure, 22
chair conformation, 33
preparation from ribose, 80
Amadori rearrangement, 179, 182
mechanism, 180
Amino acids, L-, browning reactivity, 189
catalysis of mutarotation, 100
dextrorotation, 25
dual role in carbonyl-amine reactions, 179
flavors from browning reactions, 184–185
sweet taste, 113
sweetness of enantiomers, 118
Amino sugars, negative Molish test, 76
occurrence, 46
preparation, 86
Ampholytes, sugars, 96–97
water, 95

Amylase, absence, 136
pancreatic, 128
salivary, 128
Amylases, α-, 195
β-, 194
in liver tissue, 138
Analysis of sugars in foods, 159
Angles, glycol torsion, 38
Anhydride formation, 89, 106
Anhydrides, furanoid, 107
monomolecular, 106
of D-glucose, 106
Anhydro sugars, 47, 106
Anhydrofuranoses, 82
caramelization, 169
from sulfonate esters, 82
in processed foods, 47
Anhydrous sugars, 106
Anomeric center, reactivity, 83
Anomers, absorption bands of α- and β-, 59
configuration, 26–28
definition, 10, 35
specification, 28
Anthocyanin pigments, 47
Anticaking agents, 148
Anti-clinal glycol conformation, 38
Anti-glycol conformation, 36
Antioxidants, reducing sugars as, 79
Anti-periplanar glycol conformation, 38
Arabinose, D-, aldehydo structure, 23
chair conformation, 33
in urine, 162
molecular rotation of β-, 35
occurrence, 47
polarizing power, in presence of bisulfite, 58
Arabinose, L-, 46–47
chair conformation, 33
intestinal absorption, 130
specification of α-, 28
synergism in cataractogenic effect of sugars, 135
Artichoke, Japanese, 62
Ascorbic acid, L-, 78
Aspartyl-L-phenylalanine methyl ester, L-, 150
Asymmetry, configurational, 50
conformational, 50
definition, 18
importance of conformation, 55
Atherosclerosis, 164
Athletic nutrition, 141
ATP. *See* Adenosine triphosphate
Autocatalysis, in caramelization, 173
of mutarotation, 96
Auxoglucs, 114
Available moisture. *See* Water, activity
Axial ring substituents, 29
Axis of symmetry, Sp, 52

Baker's yeast, action on fructose, 93
Barium hydroxide, use in isomerization reactions, 103
Base catalysis, mutarotation, 96
Base cations, effect on enolization, 102
Beer, sugars, 109
types, 200
Beet sugar. *See* Sucrose
Benzaldehyde, cherry flavor, 84
Benzilic acid rearrangement, 105
Benzoate esters, 81
Benzylidene derivatives, 84
Bisulfite, inhibition of browning, 190
neutralization of rotatory power, 58
Blood sugars, 131–133
insects, 135
Boat conformations, β-D-glucopyranose, 32
bowsprit and flagpole substituents, 29
cycle of, 31
cyclohexane, 29
flexibility, 31
glucose, α-D-, 27
pyranose ring, 30–31
Böeseken projection formulas, 96
Bond angles, between contiguous carbon atoms, 28
C—O—C angle of pyranose ring, 29
Borborygmia, 134
Boric acid, in configurational assignments, 26
Bowsprit ring substituents, 29
Brown sugar, 148
Browning potential in foods, 187
Browning reactions, *See also* Nonenzymic browning reactions
chemical inhibition, 190
low and high temperature, 169
purposely initiated, 169
temperature, 187

Calcium hydroxide, glucose enolization, 102
Calories, 137
  carbohydrates, fats and proteins, 113
  dietary equation, 113
  oxygen requirement, 141
Cane sugar. *See* Sucrose
Caramel, flavor, 171
  pigment, beer, 201
    nature of, 172
Caramelan, 171–172
Caramelen, 171, 174
Caramelin, 171–172. *See also* Humin
Caramelization, catalysis by ammonia, 177
  endothermic events, 170
  pH, 173
  reactions, 169
  typical flavors, 172
Caramels, 90
Carbohydrates, absorption, 128
  clinically designed, 163
  dehydration, 76
  detection, 76
  energy pathways, 138
  estimation of total content, 159–160
  general test, 75
  intolerance, 137
  lipid interactions, 139–141
Carbon atoms, asymmetry, 19, 24
  chemical labeling, 31
  enantiomeric, 10
  nonsuperposable, 20
  penultimate, 6
  reference, 10, 24–25
  tetrahedral structure, 19
Carbonyl-amine reactions, 178-186, *See also* Maillard reaction
  aldol condensation, 182
  amino acids, reactivity, 189–190
  carbonyl compounds, reactivity, 178 179
  color formation, 169, 182–183
  flavor generation, 183–186
  initial stages, 178
Cardiac glycosides, 47
Cataractogenic sugars, 77, 134
Cellobiose, α- and β-D-, 108
Chair conformations, aldohexopyranoses, 33
  aldopentopyranoses, 33
  cyclohexane, 29
  glucopyranose, β-D-, 32
  pyranose ring identification, 30
  relations, 31
Chiral elements, 18–19, 50, 53
  families, 25, 55
  properties, 18
  specifications, 19
Chitin, 46
Chloroform, taste, 114
Chondrosamine. *See* Galactose, D-, 2-amino-2-deoxy
Chromatography of sugars, 159–161
Cis, configuration of hydroxyl groups, 26, 85
  glycol configuration, 36
Classes, enzyme, 194
Cobalamin. *See* Vitamin B_{12}
Concerted interaction, sweet taste, 118
Concerted mechanism, mutarotation, 95
Condensation reactions, 72–74
  oximes, 72
  phenylhydrazones, 72
Confectionary, maltose in, 149
Conformation, barrier to crystallization, 61
  C1 chair, aldohexopyranoses, 33
    aldopentopyranoses, 33
  1C chair of ketohexoses, 40
  cyclohexane, 29
  definition, 29
  element of chirality, 50
  specification, 35
    envelope, 36
    half-chair, 35
    pyranose boats, 32
    pyranose chairs, 13
    sofa, 35
    twist, 36
  sugars in starch, 195
Conformational analysis, 31, 54
Conformational asymmetry, equilibria, 32
  formulas, operations, 42
  instability, 93
  parameters for sugars, 54
  precedence over atomic, 55
  stability and browning, 189
Configuration, definition, 17, 29
  element of chirality, 50

Configuration (*Continued*)
prefixes, 10
relationships, 10–11
Configurational isomers, 17
labels, 20
Crude fiber, composition, 165
definition, 164
dietary, 141
Crystalline hydrates, solubility, 60
Crystallinity, uses in food technology, 157
Crystallization, glucose syrups, 149
nucleating crystals, 61
reasons for, 62
sucrose syrups, 149
Cyanohydrin reaction, 79–80
Cyclitols, 123
Cyclodextrin, 158–159
Cyclohexane, taste of di-, tri- and tetrahydroxy, 123
Cyclohexylsulfamate. *See* Sodium cyclamate

D.E. *See* Dextrose equivalents
Degradation reactions, 104–106
action of acids and bases, 104
Strecker, 183
Dehydration reactions, levoglucosan formation, 89–90
pentoses and hexoses in strong acid, 75
scheme, 90
Dental plaque, 166
Deoxy sugars, definition, 11
natural occurrence, 46
preparation, 85–86
Deoxyribonucleic acid, 143
Dextran, plasma extender, 163
Dextrin, fermentation, 198
Dextro, as chiral specification, 19
configurational diagram, 53
former meaning, 25
two-fold meaning, 20
Dextrorotation, 19
convention, 53
Dextrose equivalents, 148
Diabetes, 163
Diabetics, blood sugar levels, 131
types, 161–162
Diabetogenic monosaubarides, 150
Diarrhea, glucose syrups and, 136
osmotic, 133
sugars responsible, 134
Diastereoisomers, 24
Dicarbonyl compounds, α-, caramelization, 178
Dicarboxylic acids, 79
Dietary fiber, 141, 164–165
serum cholesterol levels, 165
Digestion of carbohydrates, 128–133
Digestive disaccharidases, lack of, 136
Diheterolevulosans from fructose, 107
structure and conformation, 107
Dihydroxy acetone, condensation with glyceraldehyde, 104
ketoses from, 39
metabolic intermediate, 46
structure, 104
synthesis of formose sugars, 104
Dimer formation, glyceraldehyde, 25
Dimeric anhydrides, 107
Dimethylformamide, solvent action, 61
Dimethylsulfoxide, solvent action, 61
Dioxane ring, 108
Disaccharidases, intestinal, 136
Disaccharides, reversion products, 108
Dissymmetry, as degraded symmetry, 18
sweet taste and, 118
Diverticulitis, 141
DNA. *See* Deoxyribonucleic acid
Dulcitol. *See* Galactitol

Eclipsed glycol conformation, 38
Electrolyte effect on mutarotation, 100
Elimination reactions, β-, 105
Embden-Meyerhof-Parnas glycolysis pathway, 138, 143
diagram, 199
EMP. *See* Embden-Meyerhof-Parnas
Enantiomers, 10, 24–26
Enediol formation, 89–90
cis and *trans*, 100–101
rearrangement, 89
1,2-, 2,3-, and 3,4-, 106
Enol forms, brown color and flavor from, 182
Enolization, and isomerization reactions, 100–104
base cations, 102
pH, 101
use in synthesis, 104
rate, 101
proportionality to hydroxyl ion concentration, 106

scheme, 89–90
in caramelization, 170
Envelope conformations, 36
Enzymes, activity, 129
amylases, 194
classes, 194
fructosidases, 194, 196
galactosidases, 194, 196–197
glucosidases, 194–195
mutarotase, 98
particulately bound, 129
Epimerization, 89
energetic barrier between glucose and mannose, 103
Epimers, 24
Equatorial ring substituents, 29
Erythritol, 1,4-anhydro, taste, 122
Erythrose, D-, 23
Esterification, 81–82
Esters, benzoate, 81
sulfonate, 82
Ethane, eclipsed conformation, 52
Ethanol, rotation of glucose in, 99
Etherification, 82
Ethers, triphenylmethyl, 83
trityl, 83
Ethyl maltol, 127
Ethylidene derivatives, 84
Exo-enzymes, 194

Fehling's solution, 159
Fermentation, 197–205
malo-lactic, 204
Fischer projection formula, 3, 21
permissible operations, 40
Fischer-Tollens projection formula, 6, 28
Flagpole ring substituents, 29
Flatulence, sugars responsible, 134
Flavor, enhancers, 127
from carbonyl-amine reactions, 184–185
Fluorescence, of brown pigments, 182
Food sugars, taste, 150
Formaldehyde, generation of formose sugars, 104
Formamide, sugar solvent, 61
Formose sugars, 104, 134
toxicity, 134
Fragmentation, 89
Fructofuranose, β-D-, concentration in solution, 93
fermentation by baker's yeast, 93
specific rotation, 63
Fructofuranosylamine, α-D-, 181
Fructopyranose, β-D-, 1C conformation, 40
fermentation by baker's yeast, 93
from glucose, 89
melting point, 64
methylene carbon atom, 119
specific rotation, 63
Fructose, D-, absorption in intestine, 128, 133
absorption in rats, 140
alternative sugar in glucose-galactose malabsorption, 137
anhydrous, 64
browning reactivity, 189
crystallization from ethanol, 64
derivatives, 1-amino-1-deoxy-, 180
1-amino-2-deoxy-, 179
1-deoxy-1-glycino-, 179
2-deoxy-, 124
2,3 anhydro-, 170
dianhydride I, 108
diheterolevulosan formation, 107
dihydrate, 64
enolization, 89, 101
formula foods, 163
from glucose, 102
gas chromatography, 94
hemihydrate, 64
in blood, 131
in plants, 50–52
intestinal absorption, 128, 133
intolerance, 163
keto form, rotatory power, 94
solution concentration, 93
molecular rotation, 35
mutarotation, 92
distribution of isomers, 93
thermal, 57, 98
pH stability, 177
reduction to sorbitol and mannitol, 77
rotatory power, 26
solubility at various temperatures, 60
specific rotation, 57
stability of crystalline forms, 64
structure, 40
sweetness, 119
concentration, 156
temperature, 155

Fructose (*Continued*)
threshold, 152
use as sweetening agent, 149
syrups, 149
Fructose, DL-, from glyceraldehyde and dihydroxy acetone, 104
Fructose, L-, sweetness, 119
Fructosidases, 93, 194, 196
Fucose, D-, *See also* Galactose, D-, 6-deoxy-
intestinal absorption, 130
in urine, 162
Fucose, L-, as anticancer agent, 166
intestinal absorption, 130
structure, 166
Fuel reserves, in man, 138
Functional group isomers, 17
Furaldehydes, formation, 89–90
Furanoses, conformational isomers, 36
mutarotation, 92
Furanosidases, 194
Furanosides, acid stability, 74
Furfural, from pentoses, 75
5-(hydroxy)-methyl-, 75, 89–90

Galactitol, 77
in cataract formation, 135
1,5-anhydro-, taste, 123
Galactobiose, 49, 196
Galactopyranose, chair (C1) conformation, 33
hydrogen bonding in, 116
Galactose, D-, action of mutarotase on, 99
aldehydo structure, 22
α-anomer, 28
melting point, 64
specific rotation, 63
β-anomer, 28
melting point, 64
specific rotation, 63
bisulfite and polarizing power, 58
crystallization of α- and β-forms, 64
derivatives, 1-phosphate in galactosemia, 161
2-amino-2-deoxy-, 86
1,2: 3,4-di *O*-isopropylidine α-D-, 85
6-deoxy-, 11
intestinal absorption, 130
in urine, 162
isomers in solution, 93
occurrence, 47
sweetness, 116, 156
thermal mutarotation, 98
Galactose, L-, molecular rotation of α-, 35
natural occurrence, 46
Galactosemia, 161
Galactosidases, α-, 194, 196. *See also* Melibiase
β-, 194, 197. *See also* Lactases
Galactosylsucroses, 48, 49. *See also* Raffinose; Stachyose; Verbascose
Galacturonic acid, 79
Gentiobiose, 108, 195
Glucans, 90, 110, 200
Glucaric acid, 78, 79
Glucitol, *See also* Sorbitol
1,4- and 1,5-anhydro-, taste, 123
Glucoamylases, 195
Glucofuranose, structure, 26
1,6-anhydro-β-D-, 107
Glucogenesis, 139
Gluconic acid, sequestering agent, 78
structure, 81
Glucono-delta-lactone, 78
Glucono-gamma-lactone, 78
Glucophores, 114
Glucopyranose, α-D-, conformational formula, 2
intrinsic reactions, 90
permissible operations with Haworth formula, 42
reaction with boric acid, 27
Glucopyranose, α-L-, permissible operations with Haworth formula, 43
Glucopyranose, β-D-, conformational formula, 2, 31
glycol conformations in, 39
reaction with boric acid, 27
Glucopyranose, β-L-, 34
Glucopyranosides, β-D-, in glycosides, 47
Glucopyranosylamine, 180
Glucosan, 169
Glucosazone, 74
Glucose, D-, aldehydo structure, 22
concentration in solution, 93
anhydrous crystals, 63
bisulfite effect on polarizing power, 58

brain requirement, 138
browning reactivity, 189
Calories from fermentation, 198
conformational formula, 3
constitution, 1–3
conversion to glycogen, 113
crystalline forms, 62–64
derivatives, α-methyl, 76, 83, 121, 125
pentaacetate, 81
1,2-anhydro-α-, 109, 170
1,6-anhydro-β-, 106
1,2 : 5,6-di-*O*-isopropylidine, 85
2-amino-2-deoxy-, 86, 181
2-deoxy-, 85
3-*O*- methyl, 129, 162
6-*O*-triphenylmethyl, 83
enolization, 101
from fructose, 81
glucose-galactose malabsorption, 137
Haworth formula, 6
in plants, 50–52
monohydrate, 63
mutarotation, 91–93
pH stability, 177
solubility, 60
specific rotation, 63
concentration and temperature, 56–57
in pyridine, 100
sweetness, 113, 126, 155–156
threshold, 152
synonyms, 1
syrups, 135–136
caramelization of, 170
composition, 148
fermentation properties, 201
in athletic nutrition, 141
rare sugars in, 136
source, 148
use in renal failure, 163
thermal mutarotation, 57
tolerance curves, 132
zig-zag model, 4
Glucose, L-, aldehydo structure, 40
intestinal absorption, 130
Glucosidases, α-, 194–196
intestinal, 129
Glucosides, methyl α- and β-D- sweetness, 126
3,6-anhydro-α-, 82
Glucosone, 3-deoxy-, 104
Glucosylamine, 179, 182
Glucuronic acid, 79
Glyceraldehyde, D-, dimer formation, 25
in synthesis of formose sugars, 104
metabolic intermediate, 46
rotatory power, 25
structure, 23-25
Glyceraldehyde, L-, 25
Glycerol, from human waste products, 134
Glycerose. *See* Glyceraldehyde
Glycine, browning reactivity, 189–190
Glycogen, as fuel reserve, 138
conversion of glucose to, 113
in yeast, 200
storage diseases, 162
Glycol conformations, 36
gauche, 36, 38
sweet taste, 117
in D-glucose, 39
staggered, 36, 38
sweetness, 117
Glycol-borate, esters, acidity, 26
reaction, 26
Glycol-splitting reactions, 72
Glycols, cleavage with lead tetraacetate, 71
Glycolysis, 46, 138
Glycone, function in glycosides, 46
Glycosans, 106
Glycosidases, intestinal, 128–129
Glycosides, definition, 12
formation of methyl-, 76
inability to mutarotate, 76
internal, 107
occurrence, 46
use as drugs, 47
Glycosidic bonds, acid hydrolysis, 74
indigestable, 136
Glycosidic linkages, acid stability of, 109
identification, 83
Glycosylamine, 179
Gulonic acid, 78
Gulose, D-, aldehydo structure, 22
chair (C1) conformation, 33
Gymnemic acid, 127

Half-chair conformations, 35
Handedness, 19

Haworth formula for glucose, 6
permissible operations, 41–42
Heart disease, 139
Hemiacetals, 83–84
Heptoses, 46
Hexonic acids, 3-deoxy-. *See* Metasaccharinic acids
Hexoses, dehydration, 75
Hexosulose, 3-deoxy-D-*erythro*-, 104
3,4-dideoxy-4-sulfo-D-*glycero*-, 190
Heyns rearrangement, 181–182
Histidine, mutarotation catalyst, 100
Holocellulose, 47
Honey, in athletic nutrition, 141
sugars, 150
sweetness, 155
Humectancy of sugars, 158
Humin, 171. *See also* Caramelin
Hydrogen bonding, hydroxyl absorption bands and, 59
in crystalline lattice of sugars, 62
intermolecular, 118
intramolecular, 117
sweet taste and, 116, 118
viscosity and, 71
Hydrogenation of ketoses, 77
Hydrolysis, enzymic, 194–197
of corn starch, 149
of galactosylsucroses, 49
of glycosides, 74
of sucrose by invertase, 93–94
of sucrose during browning, 187
of sugar esters, 82
Hydrophobicity and sweet taste, 114, 118
Hydroxyl groups, absorption bands, 59
cis, reaction of ketones with, 85
reactivity to borate, 26
esterification, 81
hydrogen bonding to ring oxygen atom, 116
importance in sweet taste, 113, 122
reactivity, 109
Hygroscopic properties of sugars, 159
Hyperuricaemia, 144

Iceing sugar, 148
Idose, D-, aldehyde structure, 22
β-, 106
chair (C1) conformation, 33
conformational instability, 61
1,6-anhydro-β-, 106
Inhibine, in honey, 142
Insulin, action, 131
serum level, 164
Intestinal amylase, 132
Intestinal glucosidases, 195
Intestinal transit time, crude fiber and, 165
Intrinsic reactions of sugars, diagram, 90
Inulin, fructose in, 48
occurrence, 48
Invert sugar, resistance to crystallization, 61
rotatory power, 147
sweetness, 155
thermal mutarotation, 57
Invertase, mammalian, 196, *See also* Sucrose; Saccharase
yeast, 94, 196
Ions, role in sugar transport, 130
Isoelectric point, caramels, 172
Isomaltose, 108, 195
Isomerase, 149
Isomerization reactions, applications, 103–104
in caramelization, 170
of glucose, fructose, and mannose, 100–104
scheme, 90
Isomers, fundamental types, 17
Isopropylidine derivatives, 85
Isosaccharinic acids, 105–106
Isosacchrosan, 170–171
Iso-sweet sugar concentrations, 152–154

Japanese artichoke, 62

Ketals, formation, 85
sensitivity to acids, 85
Ketoheptose, 39
Ketohexoses, dianhydride formation, 107
isomers, 39
Ketooctose, 39
Ketoseamines, 179
Ketoses, conversion to aldoses, 80–81
definition, 7
degradation to colored substances, 182
estimation of, 93
from dihydroxyacetone, 39

hydrogenation, 77
natural occurrence, 47
phenylhydrazones, 72
reaction with hydroxylamine, 72
Ketosis, in carbohydrate under-consumption, 163
Kojibiose, in beer, 109
Krebs cycle, 138, 143

Lactases, 129, 137, 194
Lactic acid, from action of bases on hexoses, 105
from malic acid, 204
specific rotation, 25
structure, 21
Lactones, 6, 78
Lactonization, 80
Lactose (4-*O*-β-D-Galactopyranosyl-D-glucopyranose), 48
action of mutarotase on, 99
bisulfite and polarizing power, 58
cataract incidence and, 135
concentration in milk, 48
crystalline forms, 65, 157
fasting level in serum, 162
intolerance, 136–137, 163
in urine, 162
in yoghurt, 149
mutarotation, 93
natural occurrence, 48
specific rotation of α- and β-, 63
solubility, 60
sugars mistaken for, 49
Lactulose, 166–167
Laminaribiose, 108
Laxative effect of sorbitol, 134
Lead hydroxide in isomerization reactions, 103
Lead tetraacetate, in glycol-splitting reactions, 71
Levo, as chiral specification, 19
former meaning, 25
two-fold meaning, 20
Levoglucosan (1,6-anhydro-β-D-glucopyranose), 106
from 1C conformation of β-D-glucopyranose, 90
in caramelization reactions, 170
preparation from starch, 107
specific rotation, 107
taste, 107
Levorotation, 19, 53
Levulosan, 170
Levulosans, dihetero-, 17
Limen, 152
Lipid interactions with carbohydrate, 139–141
Lobry de Bruyn-Alberda van Ekenstein transformation, 100
Lyxose, D-, aldehydo structure, 23
chair (C1) conformation, 33
natural occurrence, 47
Lyxose, L-, 33

Magnesium stearate, as anticaking agent, 148
Maillard reaction, 178–186
Malabsorption, of carbohydrates, 136
of glucose and galactose, 137
of sugars, 133–134
Malic acid, fermentation, 204
Maltase, 194, 196
Maltodextrins, and blood sugar level, 132
in glucose syrup, 149
Maltol, 127
Maltose (4-*O*-α-D-Glucopyranosyl-D-glucopyranose), 48
as reversion product, 108
crystalline properties, 66
fermentation, 198
in plants, 50
in urine, 162
isomers present at mutarotational equilibrium, 93
mutarotase, 99
natural occurrence, 48
specific rotation of β-, 63
use in foods, 149
Mannan, in yeast, 200
Manninotriose, 49, 66, 196
Mannitol, 77, 80
taste of 1,4-anhydro- and 1,5 anhydro-, 123
Mannoheptulose, 39, 150
in urine, 162
Mannonic acid, 81
Mannooctulose, D-*glycero*-, 39
Mannose, D-, aldehydo structure, 22
bitter taste of β-, 65, 113
chair (C1) conformation, 33
enolization, 101
from fructose, 81, 101
from glucose, 89, 102

Mannose (*Continued*)
intestinal absorption, 128, 130
isomer distribution at mutarotational equilibrium, 93
melting point of α- and β-, 65
natural occurrence, 47
physiological disorders from, 163
preparation of β- from α-, 65
specific rotation, 63
sweetness, 116
taste of 1-deoxy-, 124
Mannose, L-, 6-deoxy, 11
Mannosylamine, 182
Medicine, role of sugars in, 161–167
Melanoidins, 183
Melibiase, 194, 196
Melibiose, 49, 196
crystalline forms. 65
Melting points of sugars, 62
Metabolism of carbohydrate, 113 137–144
Metasaccharinic acids, formation in alkaline solution, 106
isomers possible, 105
structure, 105
Methanol, specific rotation of glucose in, 99
Methyl glycosides, 76
Microbial growth, relation to water activity, 69
Microorganisms, fermentation characteristics, 202–204
intestinal, 134
Miracle berry, 127
Mirror-image compounds, 24. *See also* Enantiomers
Moisture content, role in nonenzymic browning, 189
Molecular formulas, 17
Molecular rotation, calculation, 55
conformation and, 35
Molecular structure of sugars, metabolism and, 136
Molisch's test, 75
Monosaccharides, diabetogenic, 150
Mucilages, 165
Mucopolysaccharides, 86
Multiple group stereogeometry, 115
Mushroom sugar. *See* Trehalose
Mutarotase, 98–99
Mutarotation, as intrinsic reaction of sugars, 89, 90–100
catalysis by acid and base, 95
complex, 91–92
D-fructose
concerted mechanism, 95
distribution of isomers in pyridine, 98
electrolytes, 100
distribution of isomers, 93
equilibrium, calculation from conformational free energy, 92
factors affecting rate, 98
favorable environment for, 95
intermediates, 91
mutarotase, 98
of glyceraldehyde, 25
of sugars during melting, 98
phases, rapid and slow, 92
pseudo-acyclic intermediates, 96
pyranose-furanose interconversion, 92
pyranose-pyranose interconversion, 92
simple and complex, 91
solvents, 99
source of name, 89
step-wise mechanism, 95, 97
temperature, 98
working hypothesis, 91
Mutarotational equilibrium, rotation values for sugars, 63
Myoinositol, intestinal absorption, 130

NAD. *See* Nicotinamide adenine dinucleotide
NADP, phosphorylated nicotinamide adenine dinucleotide, 143
Neohesperidin dihydrochalcone, sweetness, 150
Newman projection formulas, 38, 96–97
Nicotinamide adenine dinucleotide, 143
Nigerose, 108, 195
in beer, 109
Nitrobenzene, 1-*n*-propoxy-2-amino-4-, sweetness, 150
Nomenclature rules, 7–12
Nonenzymic browning reactions, 169–193

Nonreducing sugars, analysis, 159
Nonsuperposability, concept of, 20
Normal chair conformations, 13
Nucleic acids, 143
Nucleosides, 142
Nucleotides, biological reactions, 143
  flavor-enhancing properties, 144

Obesity, 163
Observed rotation, 55
Oligosaccharides, definition, 12
  enzymic hydrolysis, 194–197
  galactosylsucrose series of, 48
  in foods, 48
  natural occurrence, 48
  number of monosaccharide units in, 17
Optical rotation, 50–58
  rotatory power, 55
Osazones, 73, 74
Osmotic diarrhea, 133
Osmotic pressure, analogy with the gas laws, 68
  calculated and observed for sugars, 68
  of sugar solutions, 67
Osones, 73, 74
  reduction to ketoses, 74
Oxidation of sugars, 77–79
Oximes, 72
Oxy-esters, 86
Oxygen, amount required to convert food to energy, 138

Pancreatic amylase, 132, 136
Pantothenic acid, 204
Pectin, 79, 158
Pentonic acids, 2-C-methyl. *See* Saccharinic acids
  3-deoxy-C-hydroxymethyl-. *See* Isosaccharinic acids
Pentoses, as antimetabolites, 166
  dehydration of, 75
  in crude fiber, 165
  natural occurrence, 47
Perillaldehyde *anti*oxime, sweetness, 150
Perillartine, sweetness, 150
Periodate, in glycol-splitting reactions, 71
pH, anhydride formation, 90
  change during nonenzymic browning, 187
  enolization and isomerization, 101
  glucose and fructose stability, 173, 177
  mutarotation, 98
  of small intestine, 128
  role in intrinsic sugar reactions, 90
  use in monitoring the glycol-borate reaction, 26
Phenylhydrazones, 72
  further reactions with phenylhydrazine, 74
Photosynthesis, 1
Physiological ill-effects of sugars, 134
Pigments, anthocyanin, 47
  caramel, 170–173
  melanoidin, 183
Plane polarized light, composition, 53
Plaque, dental, 166
Polarimetry in sugar analysis, 159
Polarity of solvents, mutarotation, 99
Polarizability, screw pattern of, 53
Polarized light, 25
Polymerization, as intrinsic sugar reaction, 89–90, 109–110
  caramel formation, 173
  melanoidin formation, 182
  of reversion products, 109
Polyoses, from acid reversion of sugars, 109
Polysaccharide, definition, 48
Potassium hydroxide, use in isomerization reactions, 103
Projected angles, in glycol conformations, 38
Projection formulas, Böeseken, 96–97
  Fischer, 3, 21, 40
  Fischer-Tollens, 6, 11, 28
  Newman, 27, 96–97
  Sawhorse, 38
Protein, binding with sugars, 125
Psicose. *See* Allulose
Psychophysical laws, 151
Pyranose, conformational isomers, 29–36
  definition, 11
  ring, chair and boat conformations, 30
    flexibility, 30

Pyranosidases, 194
Pyranosides, acid stability, 74
Pyrazine, 2,5 dimethyl-, 186
Pyridine, as sugar solvent, 61
  mutarotation in, 96, 100
P-4000. *See* Nitrobenzene, 1-*n*-propoxy-2-amino-4-

Racemic mixtures of conformations, 52
Raffinose, crystalline properties, 66
  fasting level in serum, 162
  hydrolysis by yeast invertase, 196
  in urine, 162
  specific rotation, 63
  structure, 49
  systematic name, 48
Rare food sugars, as body fluid constituents, 162
  definition, 133
  in glucose syrups, 136
  types, 133–135
  use in foods, 149
Reactions, intrinsic, 89–90
Rearrangements, Amadori, 179, 182
  benzilic acid, 105
  Heyns, 181–182
Rectus (R), 20, 53
Reducing power of various sugars, 160
Reducing sugars, analysis, 159
  as antioxidants, 79
  difficulty in crystallizing, 61
  oligosaccharides, 49
Reduction of sugars, 77
Reference carbon atoms, 10, 24–25
Refraction, 58
Refractive index of sugar solutions, 58
Refractometry, for routine sugar analysis, 159
Relative sweetness, nonsugars, 150
  sugars, 116, 151–157
Renal failure, 163
Respiration, 197
Reversion sugars, 108–109, 171
  in glucose syrups, 136
  linkages formed, 109
Rhamnose, L-. *See also* Mannose, L-, 6-deoxy-
  intestinal absorption, 130
  natural occurrence, 46
Ribitol, 1,4-anhydro-, taste, 122
Ribonucleic acid, 143
  deoxy-, 143
Ribose, D-, aldehydo structure, 23
  chair (C1) conformation, 33
  generation of allose and altrose, 80
  induction of hypoglycemia, 166
  in nucleotides, 143
  in urine, 162
  natural occurrence, 162
  2-deoxy-, 46, 143
Ribose, L-, 33
Ring oxygen atom, bitterness and, 124
RNA. *See* Ribonucleic acid
Rotatory power, conventions, 53–54
Roughage, dietary, 165

Saccharin, sweetness, 150
  N-β-D-glycosyl-, 121
Saccharinic acids, 89, 105–106
Saccharose, 194. *See also* Invertase; Sucrase; Sucrose
Salicin, 47
Salivary amylase, 128
Salts, effect on specific rotation, 58
Salty taste, 154
Saponification, 76
Sawhorse projection formulas, 38
Schardinger dextrins, 159
Scores for sweetness, integrated factors in, 153
Screw pattern of polarizability, 53
Sequestering agents, 78
Serum triglycerides, fructose and, 140
Sinister (S), 20
Sodium bisulfite, inhibition of browning reactions, 190
  sugar polarizing power, 58
Sodium cyclamate, sweetness, 150
Sodium ions, mediation of sugar transport, 129
Sodium pump, 129
  malfunction, 137
Sofa conformation, 35
Solubility of sugars, 60
  sugar mixtures, 61
Solvents, 61
  mutarotation, 99
Sophorose, 108
Sorbitol, 47, 80
  in diabetic foods, 149

laxative effect, 134
structure, 77
Sorbose, D-, 40
DL-, from glyceraldehyde, 104
Sour taste, 154
Spatial isomers, 17
Specific and molecular rotation, 55–58
of sugars, 63
Specific gravity of crystalline sugars, 62
Spectral properties, 58
Stachyose, as source of dietary galactose, 162
crystalline properties, 67
crystallization, 61
hydrolysis by invertase, 196
in flatulence, 134
specific rotation, 63
structure, 49
systematic name, 48
Stackburn, 169
Starch, acid hydrolysis, 74
Stereoisomerism, 17
Strecker degradation, 183
Structural formulas, permissible operations, 40
Structural isomers, 17
Sucrase, 194. *See also* Saccharase; Invertase
Sucrose, calcium phosphates, 166
caramelization, 170
commercial, purity, 148
crystalline hydrates, 66
crystalline properties, 66
dehydration, 170
fasting levels in serum, 162
hydrolysis by yeast invertase, 196
in browning reactions, 187
in plants, 50, 51, 52
in urine, 162
measurement using polarimetry, 159
mutarotation of reducing sugars, 100
selective acid hydrolysis, 159
serum cholesterol and, 164
solubility, 60
source, 48
specific rotation, 63
concentration, 56
temperature, 57
syrup, 149
systematic name, 48
threshold concentration for sweetness, 152
Sugar, acetals, as food emulsifiers, 84
surfactant properties, 84
alcohols, from free sugars, 77
naming of, 77
occurrence, 47
analysis in foods, 159
carbonates, 86
esters, hydrolysis by alkali, 82
ethers, acid-base stability, 83
food technology, 157–161
formation, 82
linkages, undigestable, 136
nitrates, 86
phosphates, 86
solvents, 61
sulfates, 86
sweetness, factors affecting, 155
impact time, 153
textural properties, 151
transport, 129
structural requirements, 130
Sugars, D-, amino, 46
and heart disease, 163
anhydro, 106
biogenesis, 1
chemical properties, 71–86
conversion to glucose in the blood stream, 113
conversion to intestinal acids, 134
distribution in plants, 50–52
extraction from foods, 160
ill effects, 134
iso-sweet concentrations, 152
malabsorption, 133
occurrence and properties, 46–88
preservative action, 70, 157
reactivity in browning, 189
reasons for varying sweetness, 154
refractive index, 58
solubility, 61
supplemental taste action, 155
sweetness scores, 116
threshold concentrations, 152
therapeutic uses, 165–167
types occurring in nature, 46
Sugars, L-, as antimetabolites, 104
biological inertness, 104
construction of formulas, 34
Sulfites, loss in stored foods, 191

Sulfonate esters, 82
Superposability and optical activity, 50
Supplemental taste action, 155
Sweetness, AH,B theory of, 113–124
  assessment, 124
  intensity, structural requirement, 151
  obliteration, 127
  of sugars, 155
  of various substances, 119, 150
  potentiation and depression of, 126
  recognition thresholds, 152
  scores, for sugars, 116
    integrated factors in, 153
    interactions, 153
    variation, 115
  synergism in sugar mixtures, 127
  theories, 114
Sweet taste, and bitterness measurement, 126
  initial chemistry, 118
  tripartite requirement, 119
Symmetry, 18
  Sp axis of, 52
Syn-clinal glycol conformation, 38
Syneresis, 158
Synergism, sweetness and, 155

Tagatose, D-, chair (1C) conformation, 40
  from galactose, 101
  mutarotation, 92
Taka-invertase, 196
Taloheptulose, D-, 39
Talose, D-, aldehydo structure, 22
  chair (C1) conformation, 33
  from galactose, 101
Tartaric acid, *meso*-, 21
Taste, 113–128
  interactions, 151
  panels, 124–125
  primary sensations, 124
Tautomers, as barrier to crystallization, 61
  definition, 17
Temperature, coefficients, pyranose-furanose equilibria, 98
  mutarotation, 98
  nonenzymic browning, 187
  sugar sweetness, 155
Tetrahedron, use in structural formulas, 21
Tetrahydropyran, 2,2-dimethyl-, 50
Thermal mutarotation, 57, 98
Threose, D-, aldehydo structure, 23
Trans, configuration of hydroxyl groups, 26
  glycol configuration, 36
Transamination. *See* Strecker degradation
Trehalase, α,α-, function of, 129, 135
  in intestine, 135
  in kidney tussue, 135
Trehalose, α,α-, (α-D-Glucopyranosyl-α-D-glucopyranoside), 48, 135
  in fungal protein foods, 135, 150
  in taste studies, 121
  in yeast, 200
  occurrence, 48
  specific rotation, 63
  sweetness, 125
Trehalose, β,β-, reversion product, 108
Twist furanose conformations, 36

UDP. *See* Uridine diphosphoglucose
Ulose, definition, 11
Unsaturated aldehydes, 178
Upper epimers. *See* Anomers
Uridine diphosphoglucose, 144
Urine, sugars in, 162
Uronic acids, 79

Verbascose, 48, 49
Viboquercitol, L-, taste, 123
  DL-, taste, 123
Vicinal glycol conformations, 36
Vinegar, 204
Viscosity of sugar solutions, 70
  sweetness, 157
Vitamin B_{12}, 144
Vitamin C. *See* Ascorbic acid, L-

Water, action as an ampholyte in mutarotation, 95
  activity, of sugar solutions, 69
  in yield of glycosylamines, 179
  role in nonenzymic browning, 189

Xylan, 47
Xylitol, D-, in cataract formation, 135
  in diarrhea, 134

physiological disorders, 163
1,4-anhydro-, taste, 122
Xylose, D-, aldehydo structure, 23
chair (C1) conformation, 33
intestinal absorption, 130
in urine, 162
natural occurrence, 47
specific rotation of α- and β-, 63
use in diagnosis of sugar malabsorption, 162
Xylose, L-, 33

Yeast, in fermentation, 198
invertase, 196
osmophilic, 70

QD321.S43
Sugar chemistry
3 1775 079217 9